PHYLOGENETIC AND PHYLOGEOGRAPHIC INSIGHTS INTO THE ORIGIN OF MADAGASCAR'S SHREWS

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Abstract

Madagascar is a biodiversity 'hotspot' about 400 km off the southeast coast of Africa. All the mammals on Madagascar have diversified from within one of four ancestral lineages (Goodman and Benstead, 2005). This is remarkable because Madagascar separated 100 million years before the divergence of most mammalian orders (Krause, 2003). With the arrival of humans on Madagascar several new species were introduced (Duplantier and Duchemin, 2003), including one or possibly two introduced species of shrews (family Soricidae) *Suncus murinus* and *Suncus madagascariensis*. Although once believed to be a subspecies of the Old World *S. etruscus, Suncus madagascariensis* is currently believed to be endemic to the island, but this has never been tested (Goodman et al., 2003). I examined the phylogeny and phylogeography of the shrews occurring on Madagascar, using the mtDNA gene ND2 and a higher-level study utilizing the 16S rRNA subunit. No phylogeographic structure was recovered across the island using ND2 for either species of shrew on Madagascar. The higher-level analysis using the 16S shows little variation between *S. madagascariensis* and *S. etruscus*. Collectively, my results strongly suggest that *S. madagascariensis* is in fact a junior synonym of the *S. etruscus* and does not warrant species status. Both species of shrews that occur on Madagascar can therefore be considered introduced.

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Introduction A Brief Review of the Natural History of Malagasy Mammals

Madagascar is a biodiversity 'hotspot' about 400 km off the southeast coast of Africa. It is the fourth largest island in the world, with an area of 226,000 square miles (about 585,000 km^2). The island's fauna is noteworthy in that 84% of the native terrestrial vertebrates and 100% of the native terrestrial mammals are endemic. Of the 879 native vertebrate species on the island, 100 are mammals that have diversified from within one of four ancestral lineages (Goodman and Benstead, 2005). This is remarkable because Madagascar separated from Africa 160 million years ago, about 100 million years before the divergence of most mammalian orders (Krause, 2003). 120 -130 million years ago, Madagascar reached its current position. Until 80 million years ago, India remained connected to Madagascar before separating and eventually colliding with Asia (Flynn and Wyss, 2003).

Fossil History

The fossil record on Madagascar is extremely limited. One reason for this poor fossil record is the lack of sedimentary soils in the tropics, which are usually needed for fossils to form (Simpson, 1952). The oldest mammalian fossils found on the island date back to the Jurassic (208 – 145mya). These fossils include a mammalian jaw (*Ambondro*) with three teeth and tribosphenic dentition, a remnant from Gondwanaland, and its closest living relatives are the monotremes (Krause, 2003). There are few notable fossils from the Late Cretaceous (144-65 mya), after Madagascar's split from Africa, including a marsupial and a gondwanathere (Flynn and Wyss, 2003). No fossils exist of the extant taxa on the island that predate the Pleistocene. This lack of fossil evidence is problematic because molecular data (Kumar and Hedges, 1998; Springer et al., 2003) and some fossil data (Archibald, 1996; Kumar and Hedges, 1998) date the

origin and divergence of most mammalian orders to the Cretaceous. Molecular studies on Malagasy mammals have now filled in many of the gaps that lack fossil evidence, but fossils will ultimately strengthen molecular data and the dates associated with them. The start of the Tertiary (65 mya), after the extinction of the non-avian dinosaurs, is when some mammalian orders are thought to have diversified (Springer et al., 2003). On Madagascar the fossil record for this pivotal time in mammalian history is all but unknown. The Pleistocene is when the next notable land mammal deposits are found on Madagascar. These subfossil sites from across the island have yielded subfossils of extinct species of lemurs, pygmy hippos, and other mammals (Goodman et al., 2003). This 80 million-year gap in the fossil record is the predicted time span in which the current living terrestrial mammals are thought to have colonized the island. The lack of fossil data during the time when mammals are suspected to have radiated makes understanding their arrival on Madagascar difficult. With no real fossil evidence of when they may have arrived, colonization of Madagascar by terrestrial mammals is an open-ended question. The increased use of phylogenetics has allowed scientists to investigate further the origins of mammals on Madagascar.

Mammalian Colonization

Phylogenetic studies suggest that terrestrial mammals (carnivores, primates, rodents and tenrecs) colonized Madagascar via overwater dispersal (Dene et al., 1976; Jansa and Carleton, 2003; Olson and Goodman, 2003; Poux et al., 2005; Yoder et al., 2003). A landbridge hypothesis has also been suggested. It suggested that, during the Late Jurassic and Early Cretaceous, dry land patches appeared across the Mozambique Channel creating a landbridge (McCall, 1997). Problems arise in this hypothesis because there is very little taxonomic overlap in the mammal

faunas of Africa and Madagascar. This asymmetry between taxa is not usually seen when landbridges were present in the past. In G.G. Simpson's seminal 1940 paper, he noted that landbridges don't permit only one type of species or group to cross, travel in one direction, or transport completely imbalanced faunas. The existence of a landbridge probably would have resulted in a much more balanced and diverse fauna between Africa and Madagascar. Thus, most researchers believe that mammalian colonization of Madagascar occurred by overwater dispersal (e.g., Poux et al. 2005).

Mammalian colonization of Madagascar via overwater dispersal is no easy task, making the number of endemic mammals on the island all the more spectacular. There have only been six assumed crossings of the Mozambique Channel (400 km), by mammals from Africa to Madagascar, over the past 75 million years, suggesting that the probability of successfully crossing the Mozambique Channel is extremely low for any individual mammal (Simpson, 1952). For some taxa the probability is so low that dispersal is almost zero under any amount of time. Simpson (1952) described six factors that contribute to some species having a higher overwater dispersal probability, which include small size, protective coating, dormant stage, possible attachment to volant animals, and habitats of trees, brush or aquatic vegetation. Also contributing to overwater dispersal success in the case of Madagascar is the proximity of the animals' habitat to the east coast of Africa, and the habitat similarities between Africa and Madagascar. Simpson described this in terms of a 'sweepstakes' in which some animals will successfully make the crossing, some will not, and still others will never be given the chance (Simpson, 1952).

Malagasy Mammal Taxa

The six taxa that have successfully dispersed across the Mozambique Channel include two groups of extinct mammals and four extant lineages. The two extinct groups include at least three species of dwarf hippos (*Hippopotamus*), that according to carbon dating, survived until about 980 B.P. (Mahe and Sourdat, 1972). The second extinct taxon is *Plesiorycteropus*, a mammal that has been placed in its own order, Bibymalagasia (MacPhee, 1991), which fossils suggest was similar to aardvarks (MacPhee, 1991). Molecular data suggest that the four major groups of living terrestrial mammals on Madagascar (lemurs, carnivores, rodents and tenrecs) each radiated from a single colonizing species (Dene et al., 1976; Jansa and Carleton, 2003; Olson and Goodman, 2003; Poux et al., 2005; Yoder, 2003; Yoder et al., 2003).

The endemic primates are perhaps the most well known of the Malagasy vertebrates. This diverse group consists of at least 41 extant lemur species (Yoder, 2003). The monophyly of lemurs was formerly debated, but numerous molecular studies have confirmed colonization of lemurs by a single ancestor (Dene et al., 1976; Yoder et al., 1996). The six species of carnivores on the island were long considered to include members of the families Herpestidae and Viverridae. However, genetic studies have shown that Malagasy carnivores are monophyletic and derived from a single colonization (Yoder et al., 2003). The native rodents on the island are all in the murid subfamily Nesomyinae with only 22 species (Jansa and Carleton, 2003). Early understanding of this group's evolutionary history was filled with speculation, and not until molecular techniques were employed was monophyly of Malagasy rodents confirmed (DuBois et al., 1996; Jansa et al., 1999). Lastly, tenrecs are diverse and consist of at least 28 species (Jenkins and Carleton 2005; Goodman et al. 2006). The taxonomy and evolutionary history of tenrecs,

much like those of rodents, have been debated. At one time tenrecs were placed in the order Lipotyphla (with shrews, moles, golden moles, solenodons, and hedgehogs), tenrecs are now strongly supported as members of the superordinal clade Afrotheria along with the golden moles, elephant shrews, aardvarks, elephants, hyraxes, and dugongs (Springer et al., 1997). Also molecular studies have shown that tenrecs, like all other native Malagasy terrestrial mammals, are likely the result of adaptive radiation from a single colonization event.

Introduced Malagasy mammals

With the first arrival of humans on the island about 2000 years ago, several new species were introduced to Madagascar (Duplantier and Duchemin, 2003). Introduced species can and do have an effect on native Malagasy mammals. In general, native species are often outcompeted by, or can become prey to, introduced species. Island species are especially vulnerable to introduced species (Krajick, 2005). The effects of larger introduced mammals are well known (Krajick, 2005), but often the effects of smaller introduced mammals are underestimated or ignored. Black rats (*Rattus rattus*) and Norway rats (*R. norvegicus*) are two species whose effects on native taxa have been well studied. On Madagascar, *R. rattus* is strongly commensal and is believed to have arrived on Madagascar with the earliest human colonists (Duplantier and Duchemin, 2003). The earliest bone remnants of *R. rattus* on Madagascar date from between the 11th and the 14th century (Rakotozafy, 1996; Radimilahy, 1997). *Rattus norvegicus* is a more temperate species and, as a consequence, is not as broadly distributed across the island. It has the narrowest distribution of any of the introduced mammals on the island (Duplantier and Duchemin, 2003). *Rattus norvegicus* is believed to have arrived much later than *R. rattus,* but this may have been an inability to recognize the difference between the two species. These

species, as well as larger introduced species (e.g., cattle, goats, sheep, and pigs) can be the sources of introduced ticks, fleas, and lice, which are known to be vectors for diseases, such as typhus and intestinal schistosomiasis (Duplantier and Duchemin, 2003). *Rattus* is one of the better-studied introduced small mammals, but other small introduced mammals remain relatively unstudied.

Along with the rats on the island, there is at least one, and possibly two, introduced species of shrews (family Soricidae). The shrews on Madagascar are in the subfamily Crocidurinae. *Suncus murinus* is a non-native shrew, and, as an introduced species, its effects on the native species have gone largely unstudied. Determination of Madagascar's population(s) of *S. murinus* origination is complicated. It is believed to have arrived by shipping routes, much like *Rattus rattus,* by way of Arabia, the Red Sea, or Africa (Hutterer and Tranier, 1990). Like many other introduced mammals, it is a known human commensal (Hutterer and Tranier, 1990). It should be noted that recent biological surveys of Madagascar's deep forest have reported the occurrence of *S. murinus* (Goodman et al., 2003). This may suggest that it has been introduced several times (Hutterer and Tranier, 1990)

Madagascar's other shrew, *Suncus madagascariensis,* is believed to be endemic to the island, but this has never been tested (Goodman et al., 2003). Until 1993, *Suncus madagascariensis* was considered a subspecies of the more broadly distributed *Suncus etruscus* (Hutterer, 1993). Coquerel (1848) first described it as *Sorex madagascariensis.* He cited such characters as a more slender tail, smaller incisors, a very salient crown of the canine teeth and molars and a skull that is much smaller (although he reported a difference of only 1mm), in comparison to *S. etruscus. Suncus etruscus* is a widely distributed species that occurs throughout

much of Asia, Europe, the Middle East, and Northern Africa (Hutterer, 2005). Relationships of *S. etruscus* populations are unclear across its distribution. It has never been known to be commensal with humans. This is also the case with *S. madagascariensis,* which is found in the forests of Madagascar and seems to occur mostly in the drier western and southern forests (Goodman et al., 2003). It is rarely found in the humid east (Goodman et al., 2003). All of these characteristics have led to the belief that *S. madagascariensis* is both native and endemic to the island. A better understanding of *S. madagascariensis* and *S. etruscus* is needed to confirm the taxonomic status of *S. madagascariensis*. If native, it could signify the seventh natural crossing of the Mozambique Channel by mammals. If introduced, like *S. murinus,* the effects of *S. madagascariensis* on ecologically similar species may need to be evaluated. Using genetic techniques I will test the endemic and taxonomic status of *S. madagascariensis.*

Neither species of shrew that exists on the island is well understood, nor are their continental congeners. The taxonomy of the Old World shrews is complicated, and there are several unresolved relationships (Quérouil et al., 2001; Stanley and Olson, 2005). There are about 240 species of shrews currently recognized in Africa (Hutterer, 2005). To better understand the relationship of *S. madagascariensis* to its putative continental relative *S. etruscus,* ideally I would want to compare the two species phylogenetically. *Suncus murinus* can serve as a control with the expectation of little phylogenetic structure common in introduced or recently colonized species. However, *S. etruscus* tissue samples are rare, so instead I used phylogenetic and phylogeographic techniques to attempt to resolve the question of whether *S. madagascariensis* is indeed endemic or just an isolated population of *S. etruscus.*

Some of the endemic species found on Madagascar, most notably shrew-tenrecs (*Microgale* sp.), show many similarities to *S. madagascariensis*, including habitat, and body form, making shrew-tenrecs a good endemic taxon to compare to *S. madagascariensis.* Recent mtDNA studies in tenrec species have shown a consistent phylogeographic pattern of a historic separation of northern and southern populations, most notably in shrew-tenrecs and mole-tenrecs (Tenrecidae) (Olson et al., 2004). These phylogeographic patterns have also been found in several other native Malagasy vertebrates as well (Yoder et al., 2005). I examined the phylogeny and phylogeography of the shrews occurring on Madagascar, using the mtDNA gene NADH dehydrogenase subunit 2 (ND2). There are three scenarios that could explain the occurrence of shrews on Madagascar. First, their presence could be the result of human introduction that occurred one or more times within the past 2,000 years. This would be evident in the absence of phylogeographic structure among populations, and this is expected for *Suncus murinus.* The second possibility is that *S. madagascariensis* colonized Madagascar naturally. If this took place recently, e.g., in the last 2000 years, we would expect little, if any, phylogeographic structure. Distinguishing between human introduction and natural colonization within the last 2000 years would be difficult, at best, and most likely impossible. Lastly, if the natural colonization occurred sometime before the arrival of humans 2000 years ago and with enough time for geographically structured DNA mutations to occur via isolation by distance, we would expect to recover phylogeographic structure among the populations on Madagascar that are similar to patterns observed in mole-tenrecs and shrew-tenrecs.

My research found no phylogeographic structure and very little variation across the island in the mitochondrial DNA of *S. madagascariensis.* In fact, *S. murinus* shows more variation

between the forest-dwelling and city-dwelling forms than I found for *S. madagascariensis*. A subsequent higher-level analysis using the mtDNA gene encoding the ribosomal subunit 16S showed little variation differentiation of *S. madagascariensis* from *S. etruscus*. Collectively, my results strongly suggest that *S. madagascariensis* is in fact a junior synonym of *S. etruscus* and does not warrant species status. Both species of shrews that occur on Madagascar are most likely introduced.

References

Archibald, J.D., 1996. Fossil Evidence for a late Cretaceous origin of "hoofed" mammals. Science 272, 1150-1153.

Coquerel, M.C., 1848. Súr une espèce nouvelle de musaraigne trouvée a Madagascar. Annales des Sciences Naturelles 1, 195-198.

Dene, H., Goodman, M., Prychodko, W., Moore, G.W., 1976. Immunodiffusion systematics of the primates. III. The strepsirhini. Folia Primatol (Basel) 25, 35-61.

Dubois, J.Y., Rakotondravony, D., Hänni, C., Sourouille, P., Catzeflis, F.M., 1996. Molecular evolutionary relationships of three genera of Nesomyinae, endemic rodent taxa from Madagascar. Journal of Mammalian Evolution 3, 239-259.

Duplantier, J.M., Duchemin, J.B., 2003. Introduced small mammals and their ectoparasites: a description of their colonization and its consequences. In: Goodman, S.M., Benstead, J.P. (Eds.), The natural history of Madagascar.University of Chicago Press, Chicago. 1191-1198.

Flynn, J.J., Wyss, A.R., 2003. Mesozoic Terrestrial vertebrate faunas: the early history of Madagascar's vertebrate diversity. In: Goodman, S.M., Benstead, J.P. (Eds.), The natural history of Madagascar. The University of Chicago Press, Chicago. 34-47.

Goodman, S.M., Benstead, J.P., 2005. Updated estimates of the biotic diversity and endemism for Madagascar. Oryx 39, 73-77.

Goodman, S.M., Ganzhorn, J.U., Rakotondravony, D., 2003. Introduction to mammals. In: Goodman, S.M., Benstead, J.P. (Eds.), The natural history of Madagascar. Univ. Chicago Press, Chicago. 1159-1186.

Goodman, S.M., Raxworthy, C.J., Maminirina, C.P., Olson, L.E., 2006. A new species of shrew tenrec (*Microgale jobihely*) from northern Madagascar. Journal of Zoology 270, 384-398.

Hutterer, R., 1993. Order Insectivora. In: Wilson, D.E., and D. M. Reeder (Ed.), Mammal species of the world: a taxonomic and geographic reference. Smithsonian Institution Press, Washington D.C. 69-130

Hutterer, R., 2005. Order Soricomorpha. In: Wilson, D.E., and D. M. Reeder (Ed.), Mammal species of the world: a taxonomic and geographic reference. John Hopkins University Press. Baltimore Vol. 1: 220-260

Hutterer, R., Tranier, M., 1990. The immigration of the Asian house shrew (*Suncus murinus*) into Africa and Madagascar. In: Peters, G., Hutterer, R. (Eds.), Vertebrates of the Tropics. Museum Alexander Koenig, Bonn. 309-319.

Jansa, S.A., Carleton, M.D., 2003. Systematics and phylogenetics of Madagascar's native rodents. In: Goodman, S.M., Benstead, J.P. (Eds.), The natural history of Madagascar. Univ. of Chicago Press, Chicago. 1257-1265.

Jansa, S.A., Goodman, S.M., Tucker, P.K., 1999. Molecular phylogeny and biogeography of the native rodents of Madagascar (Muridae: Nesomyinae): a test of the single-origin hypothesis. Cladistics 15, 253-270.

Jenkins, P.D., Carleton, M.D., 2005. Charles Immanuel Forsyth Major's expedition to Madagascar, 1894 to 1896: beginnings of modern systematic study of the island's mammalian fauna. Journal of Natural History 39, 1779-1818.

Krajick, K., 2005. Winning the war against island invaders. Science 310, 1410-1413.

Krause, D.W., 2003. Late Cretaceous vertebrates of Madagascar: A window into the Gondwanan biogeography at the end of the age of the dinosaurs In: Goodman, S.M., Benstead, J.P. (Eds.), In: Goodman, S.M., Benstead, J.P. (Eds.), The natural history of Madagascar. University of Chicago Press, Chicago. 40-47.

Kumar, S., Hedges, B., 1998. A molecular timescale for vertebrate evolution. Nature 392, 917-920.

MacPhee, R.D.E., 1991. Morphology, adaptations, and relationships of Plesiorycteropus, and a diagnosis of a new order of either mammals. Bulletin of the American Museum of Natural History 220, 1-214.

Mahé, J., Sourdat, M., 1972. Sur l'extinction des Vértebrés subfossils et l'aridification du climat dans le Sud-Ouest de Madagascar. Bulletin de la Société géologique de France 14, 295-309.

McCall, R.A., 1997. Implications of a recent geological investigations of the Mozambique Channel for the mammalian colonization of Madagascar. Proceedings: Biological Sciences 264, 663-665.

Olson, L.E., Goodman, S.M., 2003. Phylogeny of Madagascar's tenrecs (Lipotyphla, Tenrecidae). In: Goodman, S.M., Benstead, J.P. (Eds.), The natural history of Madagascar. Univ. of Chicago Press, Chicago. 1235-1242.

Olson, L.E., Goodman, S.M., Yoder, A.D., 2004. Illumination of cryptic species boundaries in the long-tailed shrew tenrecs (Mammalia: Tenrecidae; *Microgale*), with new insights into geographic variation and distributional constraints. Biological Journal of the Linnaean Society 83, 1- 22.

Poux, C., Madsen, O., Marquard, E., Vieites, D.R., de Jong, W.W., Vences, M., 2005. Asynchronous colonization of Madagascar by the four endemic clades of primates, tenrecs, carnivores, and rodents as inferred from nuclear genes. Syst Biol 54, 719- 730.

Quérouil, S., Hutterer, R., Barriere, P., Colyn, M., Kerbis Peterhans, J.C., Verheyen, E., 2001. Phylogeny and evolution of African shrews (Mammalia: Soricidae) inferred from 16S rRNA sequences. Mol Phylogenet Evol 20, 185-195.

Radimilahy, C., 1997. Mahilaka, an eleventh- to fourteenth-century Islamic port: The first impact of urbanism on Madagascar. In: Goodman, S.M., Patterson, B.D. (Eds.), Natural change in human impact in Madagascar. Smithsonian Institution Press, Washington, D.C., pp. 342-363.

Rakotozafy, L.M.A., 1996. Etude de la constitution du rémime alimentaire des habitants du site de Mahilaka du XIVè au XIVè siècle à partir des produits de fouillrs archéologiques. Doctorat de Troiseme Cycle. Universite d' Antananarivo, Antananarivo.

Simpson, G.G., 1940. Mammals and landbridges. Journal of the Washington Academy of Sciences 30, 137-163.

Simpson, G.G., 1952. Probabilities of dispersal in geological time. Bulletin American Museum of Natural History 99, 163-176.

Springer, M.S., Cleven, G.C., Madsen, O., de Jong, W.W., Waddell, V.G., Amrine, H.M., Stanhope, M.J., 1997. Endemic African mammals shake the phylogenetic tree. Nature 388, 61-64.

Springer, M.S., Murphy, W.J., Eizirik, E., O'Brien, S.J., 2003. Placental mammal diversification and the Cretaceous-Tertiary boundary. Proc Natl Acad Sci U S A 100, 1056-1061.

Stanley, W.T., Olson., L.E., 2005 Phylogeny, phylogeography, and geographic variation of *Sylvisorex howelli*, an endemic shrew of the Eastern Arc Mountains. Journal of Zoology. 266:341-354.

Yoder, A.D., 2003. Phylogeny of lemurs. In: Goodman, S.M., Benstead, J.P. (Eds.), The natural history of Madagascar. University of Chicago, Chicago. 1242-1247.

Yoder, D.A., Burns, M.M., Zehr, S., Delefosse, T., Veron, G., Goodman, S.M., and Flynn, J.J., 2003. Single origin of Malagasy Carnivora from an African ancestor. Nature 42, 734-737.

Yoder, A.D., Cartmill, M., Ruvolo, M., Smith, K., Vilgalys, R., 1996. Ancient single origin for Malagasy primates. Proc Natl Acad Sci USA. 93: 5122-5126.

Yoder, A.D., Olson, L.E., Hanley, C., Heckman, K.L., Rasoloarison, R., Russell, A.L., Ranivo, J., Soarimalala, V., Karanth, K.P., Raselimanana, A.P., Goodman, S.M., 2005. A multidimensional approach for detecting species patterns in Malagasy vertebrates. Proc Natl Acad Sci USA 102 Suppl 1, 6587-6594.

Chapter 1

Phylogenetic and phylogeographic insights into the origin of Madagascar's shrews

Introduction

Madagascar is an island with spectacular biodiversity and is located off the southeast coast of Africa. It is separated from Africa by the 400 km wide Mozambique Channel. It is the fourth largest island in the world, with an area of 226,000 square miles (about 585,000 km^2). The island's fauna is noteworthy because 84% of native terrestrial vertebrates and 100% of its species of terrestrial native mammals are endemic. Madagascar also contains extremely high vertebrate biodiversity, with 879 native species. Currently, only four extant orders of terrestrial mammals occur naturally on Madagascar: primates (lemurs), carnivores, nesomyine rodents, and tenrecs (Jansa and Carleton, 2003; Olson and Goodman, 2003; Yoder, 2003; Yoder et al., 2003). Two additional, now extinct, native terrestrial eutherian mammal groups are known from Madagascar, including at least three species of pygmy hippos (Order Artiodactyla) and *Plesiorycteropus* (Order Bibymalagasia), a mammal similar to the modern aardvark (MacPhee, 1991). Only six lineages of mammals have naturally colonized Madagascar, suggesting the probability of successfully crossing the Mozambique Channel is low (Simpson, 1952). The fossil record and molecular clock estimates suggest that most living eutherian orders diverged about 65 million years ago, long after Madagascar's separation from Africa 160 million years ago (Krause, 2003), indicating all endemic terrestrial mammals on Madagascar dispersed over water to the island (Poux et al. 2005). It appears that the four extant terrestrial mammal lineages colonizing Madagascar each radiated from a single ancestral lineage and have subsequently undergone spectacular radiations.

In contrast to its overall high vertebrate endemism and biodiversity, Madagascar is home to only two species of shrews; *Suncus murinus* and *Suncus madagascariensis* (Soricidae: Crocidurinae). Only *S. madagascariensis* is considered native (and endemic) to Madagascar. In contrast, about 240 species, more than half of all shrew species globally are found on continental Africa. This striking asymmetry between the shrew faunas of Madagascar and Africa is not atypical, and is observed in other groups of mammals found on continental Africa but are absent on Madagascar (Simpson, 1940).

Approximately 2000 years ago, concurrent with the arrival of humans, several mammal species purportedly were introduced to Madagascar. These included one, or possibly two, shrew species (family Soricidae) (Goodman et al., 2003). The close association between *S. murinus* (house shrew) and humans likely contributed to its introduction to Madagascar (Goodman et al., 2003). Whether *S. madagascariensis* was introduced is not well understood.

Based purely on morphology, *S. madagascariensis* was long considered a subspecies of the Old World *S. etruscus* (Heim de Balsac and Meester, 1977, Hutterer, 1993). More recently, however, *S. madagascariensis* was elevated to full species status based on geographic isolation (Eisenburg and Gould, 1984; Hutterer, 1993). *Suncus madagascariensis* is currently considered endemic to Madagascar (Goodman et al., 2003). However, there is no strong molecular or morphological evidence that *S. madagascariensis* is indeed distinct from *S. etruscus* (Goodman et al., 2003). Confusion as to the status of this species began as early as 1848 when Coquerel first described this species as *Sorex madagascariensis*. He cited such characteristics as a more slender tail, smaller incisors, a salient crown of the canine teeth and molars, and a smaller skull (although he reported a difference of only 1mm) than *Suncus etruscus* (Coquerel, 1848). *Suncus*

madagascariensis' mainland counterpart, *S. etruscus,* is widely distributed, found throughout much of Southern Asia, Europe, the Middle East, and Northern Africa. Relationships between *S. etruscus* populations are unclear across its distribution. Despite its broad distribution, it has never been known to be a human commensal. This is also the case with *S. madagascariensis*, which inhabits the undisturbed forests of Madagascar and apparently occurs, mostly in the drier western and southern forests (Goodman et al., 2003). It is rarely found in the humid eastern forests (Goodman et al., 2003). All of these characteristics have lead to the speculation that *S. madagascariensis* is endemic to the island. A better understanding of the phylogenetics of *S. madagascariensis* and *S. etruscus* is needed in order to confirm the endemic status of *S. madagascariensis* on the island. If *S. madagascariensis* is native, it would be the result of the seventh natural crossing of the Mozambique Channel by terrestrial mammals. If introduced, like *S. murinus,* the impacts of *S. madagascariensis* on ecologically similar species may need to be evaluated. Using phylogenetic methods, I will test the taxonomic status of *S. madagascariensis*.

Suncus murinus is broadly distributed across much of Asia, the Arabian Peninsula, Eastern and Northern Africa, and Madagascar. Determining the original origin of *S. murinus* is complicated. *Suncus murinus* is believed to have arrived by a similar route to *Rattus rattus* via Arabia, the Red Sea, or Africa (Hutterer and Tranier, 1990). As indicated above, *Suncus murinus* is a human commensal, which directly contributed to its introduction, possibly several times (Hutterer and Tranier, 1990). Biological surveys have demonstrated that *S. murinus* has penetrated Madagascar's deep forests (Goodman et al., 1996). The forest-dwelling *S. murinus* may be the result of an earlier introduction to the island, separate from more recent introductions (Hutterer and Tranier, 1990). Yamagata et al. (1990) suggested a similar scenario for

populations of *S. murinus* in Asia. Commensal populations of *S. murinus* showed no variation in their mtDNA whereas non-commensal, potentially older, populations mtDNA were more variable. Molecular studies are the next step in estimating the number of times the species has been introduced to Madagascar.

Confusion as to the origins of these two species is not confined to Madagascar but is applicable to the entirety of their known distributions in the Old World. To better understand the relationship of *S. madagascariensis* to its putative continental relative *S. etruscus*, the two species should be included in phylogenetic analyses with other close relatives. However, African shrew taxonomy contains several unresolved relationships, including many between species and genera (Quérouil et al., 2001; Stanley and Olson, 2005). There are approximately 240 species of shrews currently recognized in Africa (Hutterer, 2005). However, such studies are complicated by the rarity of tissue samples available for *S. etruscus*. Nonetheless, phylogenetic and phylogeographic techniques are used in this study in an attempt to resolve the question as to whether *S. madagascariensis* is endemic or introduced.

Some of the endemic species found on Madagascar, most notably shrew-tenrecs (Tenrecidae), show similarities to *S. madagascariensis*. These include habitat, lifestyles, and body forms, making shrew-tenrecs good endemic Malagasy species to compare to *S. madagascariensis*. A recent mtDNA study of long-tailed shrew tenrecs uncovered a phylogeographic pattern suggesting a historic separation between northern and southern populations (Olson et al., 2004). These phylogeographic patterns have also been found in several other native Malagasy vertebrates (Yoder et al., 2005). This study examines the phylogeny and phylogeography of the shrews occurring on Madagascar using the mtDNA gene

NADH dehydrogenase subunit 2 (ND2 hereafter). A mitochondrial DNA study is the most practical in evaluating the phylogeography of *S. madagascariensis* and *S. murinus*. This gene has been shown to be a good marker for analyses within species of shrew-tenrecs and other crocidurine shrews (Goodman et al., 2006; Olson et al., 2004; Stanley and Olson, 2005).

Three scenarios could explain the occurrence of shrews on Madagascar. First, the presence of shrews could be the result of human introductions that occurred at some time over the past 2,000 years. This would be supported by the absence of phylogeographic structure among populations, and is expected for *S. murinus*. The second possibility is that *S. madagascariensis* colonized Madagascar naturally. If this took place in the last 2,000 years, we would expect little if any phylogeographic structure. Distinguishing between human introduction and natural colonization within the last 2,000 years is difficult and most likely impossible because there is no way to determine how *S. madagascariensis* got to the island in the first place. Finally, if the natural colonization occurred sometime prior to the arrival of humans 2,000 years ago, and with enough time for mutations to accumulate in the mitochondrial genome of different populations then phylogeographic structure is expected among the populations on Madagascar. This would be similar to that observed in ecologically similar small mammals (e.g. shrew-tenrecs) with comparable distributions.

Methods

DNA was isolated from 37 samples (23 *S. madagascariensis* and 14 *S. murinus)* of frozen or buffered muscle or kidney tissues using the PureGene kit (Gentra Systems, Inc, Minneapolis, MN, USA) with reference to the animal tissues protocol. Primers used for ND2 (1044 bp) were the external primers MET-1 and Trp-2 (Olson et al. 2004), and the internal primers ND2-SF1,

ND2-SR1 (Stanley and Olson, 2005), and ND2-SR2 (5' TGGGCAATGGATGAATATGCC 3') were made specifically for *S. madagascariensis*. PCR amplifications were performed in 25 µl, reactions including 5 µl unquantified DNA in a 1:50 dilution from the original extraction, 1 µl of 10 mM of primer, 2.5 µl 10X buffer with 15 mM $MgCl_2$, 0.25 µl of 5U/ µl Taq polymerase (Promega, Madison WI, USA), and 0.5 µl dNTPs (100mM). An additional 1 µl of 25mM $MgCl_2$ and water was added to bring the final magnesium concentration to 2.5 mM. Thermal cycling parameters included 2 minutes at 94 °C, followed by 30 cycles of 20 seconds at 94 °C, 15 seconds at 55 °C, and 60 seconds at 72 °C, with a two minute final extension at 72 °C. When bands of the appropriate size were obtained, the remaining PCR product was purified using 1.25 µl Exo-SAP It (USB Inc, Cleveland, OH, USA) per 5 µl of PCR reaction. Unquantified aliquots of the purified PCR products were cycle-sequenced for both strands using 1 µl purified DNA, 0.5 µl BigDye terminator 3.1 (Perkin-Elmer, Boston, MA, USA), 1.75 5X buffer, 1 µl primer, and water was added to make a total volume of 10 µl per reaction. Sequencing reactions were purified using Sephadex (MP Biomedicals, Inc., Solon, OH, USA) according to the manufacturer's instructions. Samples were electrophoresed on an ABI 3100 automated sequencer (Perkin-Elmer). Resulting sequence contigs were aligned using Sequencher 4.2 (Genecodes, Ann Arbor, MI). The resulting sequences were deposited in GenBank under accession numbers EF507210-EF507246.

Data Analysis. Samples of *S. madagascariensis* and *S. murinus* were from several localities across the island and the Philippines (*S. murinus* only) (Appendix 2 and Figure 1). Additional Crocidurinae shrew ND2 sequences were taken from GenBank for comparison and outgroups (Appendix 1). *Sylvisorex vulcanorum* was used as an outgroup to root all trees based

on results from Stanley and Olson (2005). Resulting sequences were aligned by eye with reference to the translated amino acid sequence in MacClade 4.0 (Maddison and Maddison, 2000)

Phylogenetic analyses. Phylogenetic analyses were performed under maximum parsimony (MP) and maximum likelihood (ML) using PAUP* 4.0 (Swofford, 2003). All characters were treated as unordered and equally weighted in the MP tree searches. Heuristic tree searches were conducted using stepwise addition (100 random addition sequences) and the TBR branch-swapping algorithm. Likelihood parameters were estimated using the program ModelTest 3.5 (Posada and Crandall, 1998). Parameter values were fixed in a search employing 100 heuristic replicates with TBR branch swapping in PAUP*. Bootstrap support was evaluated from 100 pseudoreplicates using the TBR branch-swapping algorithm.

Bayesian posterior probabilities were estimated to assess nodal support using MrBayes (Ronquist and Hueslenbeck, 2005). Four MCMC chains (three heated, one unheated) were allowed to proceed for 5 million generations, and trees were sampled every 100 generations. Samples before likelihood values converged on a stable topology were discarded as burn-in. All remaining trees were used to estimate posterior probabilities.

Results

Three haplotypes were recovered from both *S. murinus* and *S. madagascariensis*. Redundant sequences were excluded, which therefore resulted in three *S. madagascariensis* and three *S. murinus* sequences. These sequences were included in all analyses along with sequences from GenBank. All sequences were identical in length. Of the 1044 bps of ND2 in all ingroup

taxa, 477 were constant, 104 were variable and 463 were parsimony-informative. Uncorrected distances ranged from 0 - 0.3% within *S. madagascariensis*, 0 - 2.1% within *S. murinus,* and 16% - 17.19% between species. Each of the three haplotypes from *S. madagascariensis* differed at a single position. Of the 23 specimens sampled, only two differed from the predominant haplotype. Three haplotypes were recovered for *S. murinus*, two from Madagascar and one from the Philippines.

The heuristic tree search, under maximum parsimony, resulted in a tree that was 1,597 steps, shown in Figure 2 with bootstrap support values. The preferred model for the maximum likelihood analysis was the GTR with a gamma distribution of 0.8207, and the proportion of invariant sites was equal to 0.3350 with a rate matrix of 0.2387, 6.5026, 0.2387, and 0.6983. The single ML tree (-lnL=7985.82) and corresponding ML bootstrap values are shown in Figure 2. Likelihood scores converged on a stable value before 125,000 generations in the Bayesian analysis. The first 200,000 generations were therefore conservatively excluded as burn-in, and the resulting posterior probabilities are shown in Figure 2.

All analyses resulted in a similar topology to that found in Stanley and Olson (2005). All *S. murinus* samples clustered together with high bootstrap support. The Madagascar samples formed a clade with high bootstrap support and the sample from the Philippines fell basal to the two Malagasy samples. All *S. madagascariensis* samples formed a monophyletic clade.

Discussion

The data shown had minimal variation in both species of shrews on Madagascar and phylogeographic structure. Of the 23 tissue samples there were only three variant sequences

among *S. madagascariensis*, and these differed at only a single position. Only two haplotypes were recovered on the island for the 14 *S. murinus* samples, and these were more variable (0.9% uncorrected pairwise difference) than the single base differences (0.3% uncorrected pairwise difference) in the *S. madagascariensis* samples.

All sequences generated for *S. murinus* from the Philippines were identical. All Malagasy *S. murinus* sequences were identical with one exception. This specimen (GenBank accession no. **AY691841**) was the only sample collected in undisturbed forest, while all others were collected from human-inhabited areas. This may suggest that the two haplotypes are the result of separate introductions. However, more samples are necessary to confirm this. If this scenario is correct the first introduction of *S. murinus* may be from early trading routes established sometime during the 11th and 14th centuries (Hutterer and Tranier 1990), and the second introduction might be from more recent human activity. The lack of genetic diversity at this locus among *S. murinus* across the island may be the result humans inadvertently transferring *S. murinus* across the island, causing high levels of gene flow. In an mtDNA study using restriction enzymes, *S. murinus* on Japanese and Indonesian islands, Yamagata et al. (1990) detected no variability. However, *S. murinus* from areas of suspected older populations (Sri Lanka) unassociated with humans were more variable (Yamagata et al. 1990). The single *S. murinus* from undisturbed forest in Montage d' Ambre, Madagascar was collected near a suspected early shipping port (Goodman et al., 1996). This may be an area associated with *S. murinus'* early introduction to the island. All captured records of *S. murinus* in Arabia, the Red Sea, Africa and Madagascar are located in areas near historic Arab trading routes (Hutterer and Tranier, 1990). Bone remains from *S. murinus* have been found in Anjohibe Cave near

Mahilaka, which is another suspected early shipping port in Madagascar (Burney et al, 1997; Radimilahy, 1997). Collectively, these results and previous observations suggest that *S. murinus* was introduced to Madagascar via ships.

Heim de Balsac (1972) suggested that if *S. murinus* came to inhabit the native forest it would compete with/or prey on, the native species. However, in the large forested area of Ambohitantely, where *S. murinus* is present, it apparently has not outcompeted the native shrew-tenrecs (*Microgale* spp.)(Goodman et al., 2003).

Ectoparasites of *Rattus* spp. have traditionally transmitted *Yersinia pestis,* the etiologic agent of plague, which is a significant public health concern in some parts of Madagascar. *S. murinus* may play an important role as a reservoir for the plague in Mahajanga, Madagascar, where it is the primary introduced species, as it can harbor the flea species known to transmit the plague bacteria (Duplantier and Duchemin, 2003). *Suncus murinus* may be more recently introduced in these areas than the *S. murinus* found in undisturbed forest. Eliminating *S. murinus* as a source of the plague may be difficult, as Seymour et al. (2005) found that six months after an extensive eradication effort on the Ile aux Aigrettes off the coast of Mauritius that *S. murinus* numbers began to rebound rapidly.

The data presented in this study suggested that *S. madagascariensis* arrived recently to the island and therefore is unlikely to be endemic or native to the island. A phylogeographic study of shrew-tenrecs, which are endemic and ecologically similar to shrews, showed strong phylogeographic structure, with deeply divergent haplotype clades corresponding to historically northern and southern populations (Olson et al., 2004). Other endemic vertebrate species have also shown similar phylogeographic patterns across Madagascar (Yoder et al., 2005). If *S.*

madagascariensis colonized naturally long before the arrival of humans, it would be expected to have experienced the same environmental pressures as shrew-tenrecs, and therefore, to express strong phylogeographic patterns among populations. Phylogeographic patterns have not only been found in native Malagasy species but also in African crocidurine shrews. *Sylvisorex howelli* is an endemic species that inhabits the Eastern Arc Mountains of Tanzania. The species is highly variable at the ND2 locus not only between mountain populations but also along an elevational gradient within single mountain populations (Stanley and Olson, 2005). *S. howelli* showed divergences between 1.1% and 8.1% (Stanley and Olson, 2005), which is much greater than either of the shrew species present on Madagascar.

As a result of the data presented in this study, *S. madagascariensis* should not be considered an endemic species or the seventh documented natural colonization of Madagascar by terrestrial mammals. The question remains: how did an apparent non-commensal species come to inhabit the forests across Madagascar without developing any significant genetic variation? *Suncus madagascariensis* is very small; therefore, island-wide dispersal under its own power may seem unlikely. However, pygmy shrews, *Sorex minutus* (similar in size to *S. etruscus*) are known to travel between 60-80 meters a day (Churchfield, 1990). At that rate it would take only 69 years for the species to span the island north to south, assuming the shrew was deposited at either the southern or northern end of the island. If *S. madagascariensis* was introduced by the first humans on the island 2000 years ago, it would only need to travel about 2.5 meters a day to cover the entire island by now. Nogales et al. (2006) suggested that *S. etruscus* may be invasive in the Canary Islands and, therefore, may have more interactions with humans than previously

thought. In a scenario resembling that of Madagascar the date of its introduction to the Canary Islands is unknown and nothing is known of its effects on the native fauna (Nogales et al., 2006).

The results of this study show little variation among *S. madagascariensis*. However, they do raise two questions. First, how did *S. madagascariensis* arrive on the island? Second, did humans facilitate *S. madagascariensis* arrival? Further sampling from across the geographic range of *S. etruscus* would also give some insight as to the origin of *S. madagascariensis*. An analysis of 16S has shown that there is little divergence (1.58% uncorrected pairwise distance) between *S. madagascariensis* and *S. etruscus* (see chapter 2). This suggests that *S. madagascariensis* should be synonomized with *S. etruscus*. Likewise, more samples of *S. murinus* will also shed additional light on its origins. The effects of *S. madagascariensis* and *S. murinus* on native populations may need to be evaluated, especially where they may be competing with native small mammals and/or harboring diseases to which humans and native taxa may be vulnerable.

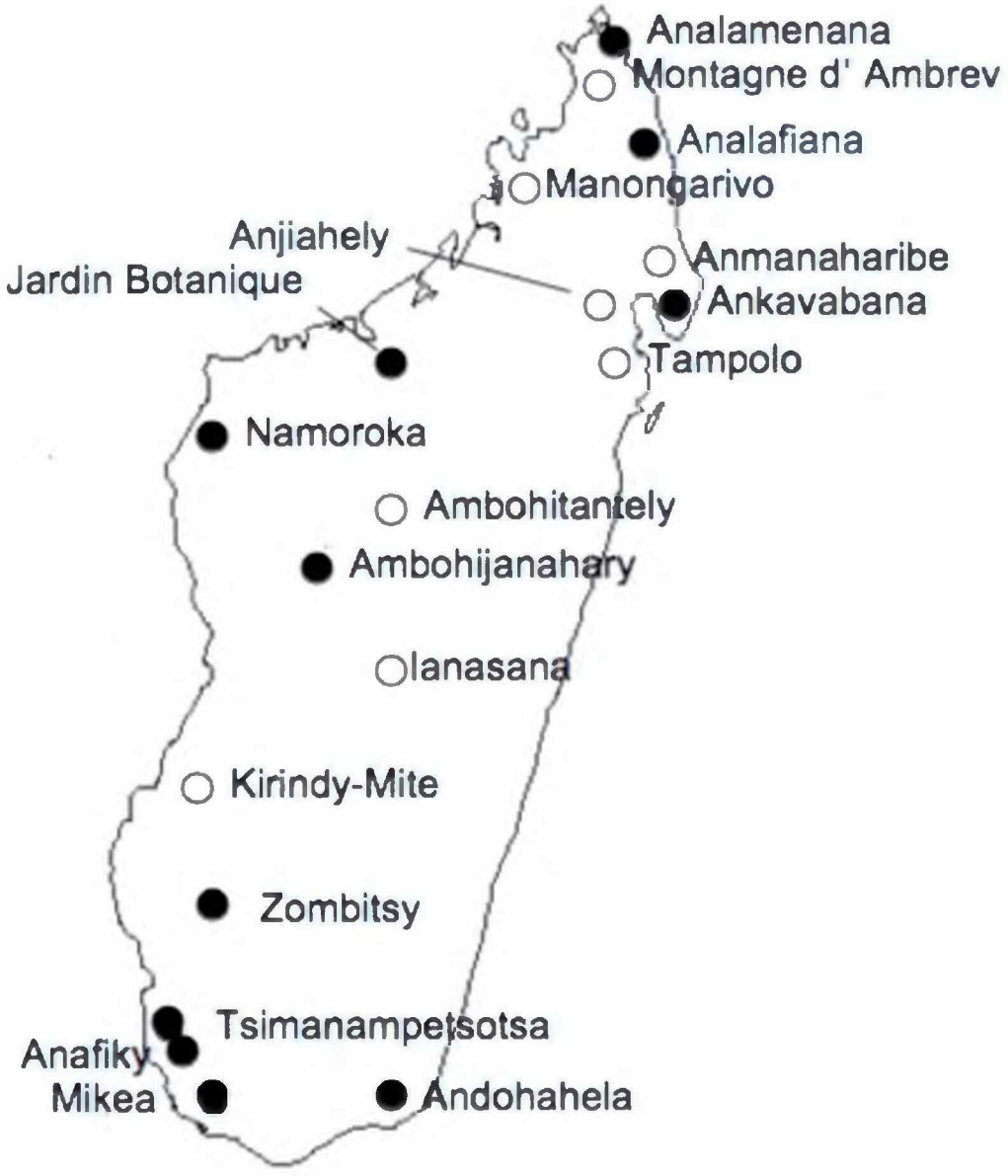


Figure 1.1: Map of Madagascar, with collecting localities for specimens analyzed in this study. For exact localities see Appendix 1. *Suncus murinus* localities are denoted with an open circle and *S. madagascariensis* are denoted with closed circles.

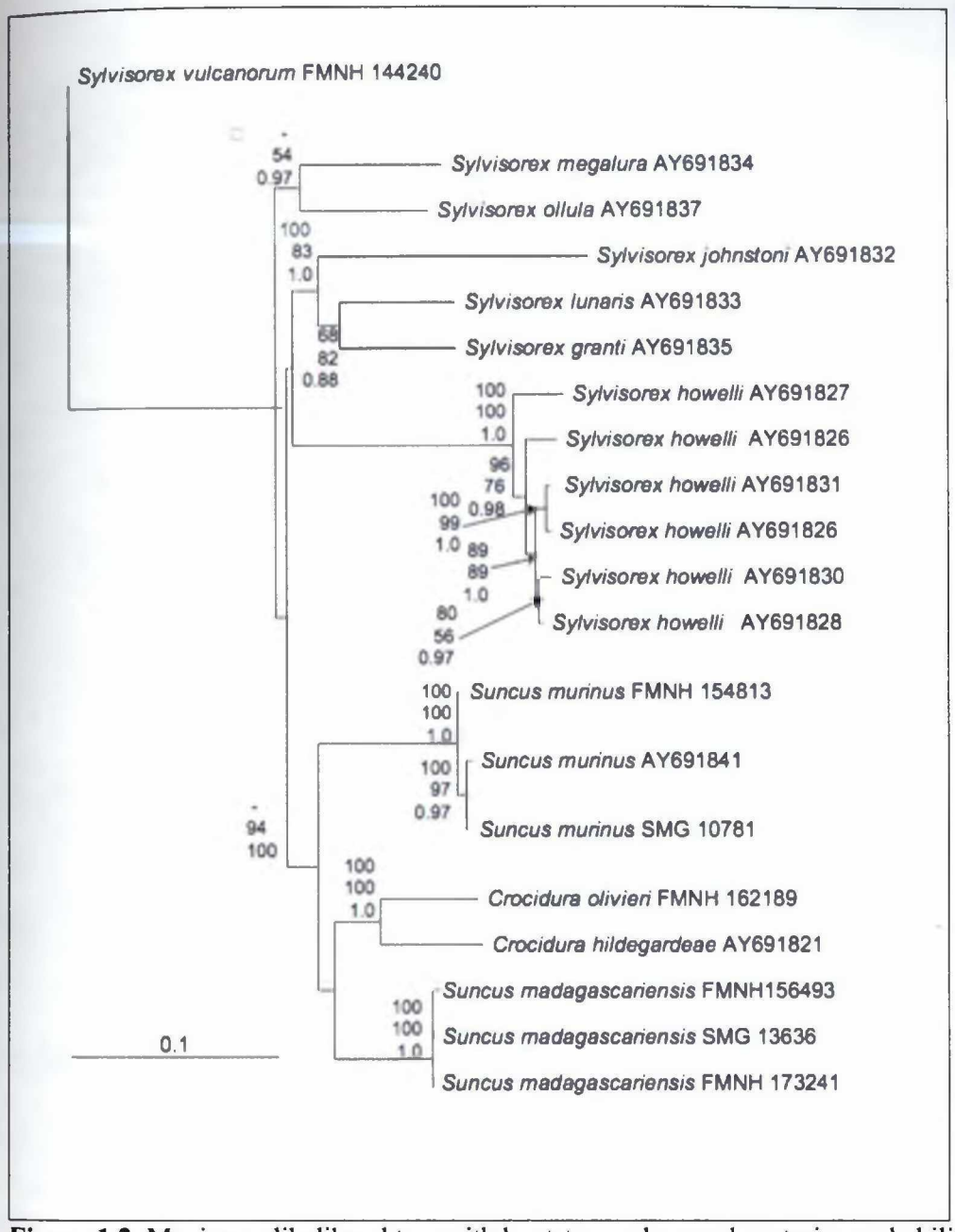


Figure 1.2: Maximum likelihood tree with bootstrap values and posterior probabilities. First and second numbers represent maximum parsimony (MP) and maximum likelihood (ML) bootstrap support values (respectively) after 1000 pseudoreplicates, followed by Bayesian posterior probabilities. Values denoted with dashes represent values < 50%. All duplicate haplotypes have been removed. Abbreviations and numbers are FMNH catalogue numbers. Specimens not noted with FMNH number are as-yet uncatalogued and were collected by Steven M. Goodman (SMG) and numbers following initials are field numbers.

References

Burney, D.A., 1997. Theories and facts regarding Holocene environmental change before and after human colonization. In: Goodman, S.M., Patterson, B.D. (Eds.), Natural change and human impact in Madagascar. Smithsonian Institution Press, Washington D.C., pp. 75-89.

Churchfield, S., 1990. The natural history of shrews, A & C Black, London.

Coquerel, M.C., 1848. Sûr une espèce nouvelle de musaraigne trouvée a Madagascar. Annales des Sciences Naturelles 1, 195-198.

Duplantier, J.M., Duchemin, J.B., 2003. Human diseases and introduced small mammals. In: Goodman, S. M., Benstead, J.P. (Eds.), The natural history of Madagascar. Univ. Chicago Press, Chicago. 1191-1198.

Eisenburg, J.F., Gould, E., 1984. The insectivores. In: Jolly, A., Oberle, P., Albignac, R. (Eds.), Madagascar. Pergamon Press, Oxford, pp. 155-165.

Goodman, S.M., Andrianarimisa, A., Olson, L.E., Soarimalala, V., 1996. Patterns of elevational distribution of birds and small mammals in the humid forests of Montagne d' Ambre, Madagascar. Ecotropica 2, 87-97.

Goodman, S.M., Ganzhorn, J.U., Rakotondravony, D., 2003. Introduction to mammals. In: Goodman, S.M., Benstead, J.P. (Eds.), The natural history of Madagascar. Univ. Chicago Press, Chicago. 1159-1186.

Goodman, S.M., Raxworthy, C.J., Maminirina, C.P., Olson, L.E., 2006. A new species of shrew tenrec (*Microgale jobihely*) from northern Madagascar. J. Zool. 270, 384-398.

Heim de Balsac, H., 1972. Insectivores. In: Battisitini, R., Richard-Vindard, G. (Eds.), Biogeography and ecology in Madagascar. W. Junk, The Hague, pp. 629-660.

Heim de Balsac, H., Meester, J., 1977. Order Insectivora. In: Meester, J., Setzer, H.W. (Eds.), The mammals of Africa: an identification manual. Smithsonian Institute, Washington D.C., pp. 1-29.

Hutterer, R., 1993. Order Insectivora. In: Wilson, D.E., and D. M. Reeder (Ed.), Mammal species of the world: A taxonomic and geographic reference. Smithsonian Institution Press, Washington D.C. 69-130.

Hutterer, R., 2005. Order Soricomorpha. In: Wilson, D.E., and D. M. Reeder (Ed.), Mammal species of the world: a taxonomic and geographic reference. Johns Hopkins University Press. Baltimore. Vol. 1: 220-260.

Hutterer, R., Tranier, M., 1990. The immigration of the Asian house shrew (*Suncus murinus*) into Africa and Madagascar. In: Peters, G., Hutterer, R. (Eds.), Vertebrates of the tropics. Museum Alexander Koenig, Bonn. 309-319.

Jansa, S.A., Carleton, M.D., 2003. Systematics and phylogenetics of madagascar's native rodents. In: Goodman, S.M., Benstead, J.P. (Eds.), The natural history of Madagascar. Univ. of Chicago Press, Chicago. 1257-1265.

Krause, D.W., 2003. Late Cretaceous vertebrates of Madagascar: A window into the Gondwanan biogeography at the end of the age of the dinosaurs In: Goodman, S.M., Benstead, J.P. (Eds.), The natural history of Madagascar. University of Chicago Press, Chicago. 40-47.

MacPhee, R.D.E., 1991. Morphology, adaptations, and relationships of Plesiorycteropus, and a diagnosis of a new order of either mammals. Bull. Am. Mus. Nat. Hist. 220, 1-214.

Maddison, D.R., Maddison, W.P., 2000. MacClade 4. Analysis of phylogeny and character evolution. Sinauer Associates, Sunderland, MA.

Nogales, M., Rodriguez-Luengo, J.L., Marrero, P., 2006. Ecological effects and distribution of non-native mammals on the Canary Islands. Mamm. Rev. 36, 49-65.

Olson, L.E., Goodman, S.M., Yoder, A.D., 2004. Illumination of cryptic species boundaries in the long-tailed shrew tenrecs (Mammalia: Tenrecidae; *Microgale*), with new insights into geographic variation and distributional constraints. Biol. J. Linn. Soc. 83, 1- 22.

Olson, L.E., Goodman, S.M., 2003. Phylogeny of Madagascar's tenrecs (Lipotyphla, Tenrecidae). In: Goodman, S.M., Benstead, J.P. (Eds.), The natural history of Madagascar. Univ. of Chicago Press, Chicago. 1235-1242.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817-818.

Poux, C., Madsen, O., Marquard, E., Vieites, D.R., de Jong, W.W., Vences, M., 2005. Asynchronous colonization of Madagascar by the four endemic clades of 719-730.

Quérouil, S., Hutterer, R., Barriere, P., Colyn, M., Kerbis Peterhans, J.C., Verheyen, E., 2001. Phylogeny and evolution of African shrews (Mammalia: Soricidae) inferred from 16S rRNA sequences. Mol. Phylogenet. Evol. 20, 185-195.

Radimilahy, C., 1997. Mahilaka, an eleventh- to fourteenth-century Islamic port: The first impact of urbanism on Madagascar. In: Goodman, S.M., Patterson, B.D. (Eds.), Natural

change in human impact in Madagascar. Smithsonian Institution Press, Washington, D.C., pp. 342-363.

Ronquist, F., Hueslenbeck, J.P., 2005. MrBayes: Phylogenetic inference under mixed models, 3.1.1., Uppsala, Sweden.

Seymour, A., Varnham, K., Roy, S., Harris, S., Bhageerutty, L., Church, S., Harris, A., Jennings, N.V., Jones, C., Khadun, A., Mauremootoo, J., Newman, T., Tatayah, V., Webbon, C., Wilson, G., 2005. Mechanisms underlying the failure of an attempt to eradicate the invasive Asian musk shrew *Suncus murinus* from an island nature reserve. Biol. Conservation 125, 23-35.

Simpson, G.G., 1940. Mammals and land bridges. J. Washington Acad. Sci. 30, 137-163.

Simpson, G.G., 1952. Probabilities of dispersal in geological time. Bull. Amer. Mus. Nat. Hist. 99, 163-176.

Stanley, W.T., Olson, L.E., 2005. Phylogeny, phylogeography, and geographic variation of *Sylvisorex howelli*, an endemic shrew of the Eastern Arc Mountains. J. Zool. 266 341-354.

Swofford, D.L., 2003. PAUP*: Phylogenetic analysis using parsimony (and other methods). Sinauer Associates, Sunderland, MA.

Yamagata, T., Tanaka, Y., Ishikawa, A., Namikawa, T., Tomita, T., 1990. Genetic-relationship among the musk shrews, *Suncus murinus* (Insectivora), Inhabiting islands and the continent based on mitochondrial-DNA Types. Biochemical Genetics 28, 185-195.

Yoder, A.D., 2003. Phylogeny of lemurs. In: Goodman, S.M., Benstead, J.P. (Eds.), The natural history of Madagascar. University of Chicago, Chicago. 1242-1247.

Yoder, A.D., Olson, L.E., Hanley, C., Heckman, K.L., Rasoloarison, R., Russell, A.L., Ranivo, J., Soarimalala, V., Karanth, K.P., Raselimanana, A.P., Goodman, S.M., 2005. A multidimensional approach for detecting species patterns in Malagasy vertebrates. Proc. Natl. Acad. Sci. USA. 102 Suppl 1, 6587-6594.

Yoder, D.A., Burns, M.M., Zehr, S., Delefosse, T., Veron, G., Goodman, S.M., and Flynn, J.J., 2003. Single origin of Malagasy Carnivora from an African ancestor. Nature 42, 734-737.

Chapter 2 Phylogenetics of African shrews, with emphasis on Madagascar's endemic shrew *Suncus madagascariensis*

Introduction

Shrews (Soricidae) are the third most speciose extant family of mammals, with 376 described extant species in 26 genera (Hutterer, 2005). Shrews are divided into 3 subfamilies and are found on every continent except Antarctica and Australia. Soricinae is distributed in the Northern Hemisphere, Crocidurinae occurs in the Old World (mostly in Africa) and Myosoricinae is found only in Africa. Shrews are difficult to differentiate based on morphological features, such as body size, pelage, skull and tooth shape (Heim de Balsac and Lamotte, 1956, 1957; Meester, 1953). These characters are often not clearly defined, causing many taxonomic uncertainties, particularly at the genus and species levels. Reconstructing the evolutionary history of shrews is exceptionally difficult and has relied mostly on studies of extant species due to a limited fossil record (Butler, 1998).

More than half, 240, of the known species of shrews occur in Africa (Hutterer 2005). In contrast, Madagascar is home to only two species of shrews, *Suncus murinus* and *S. madagascariensis.* This striking asymmetry between the shrew faunas of Africa and Madagascar is not atypical, since several groups of mammals present on Africa are absent on Madagascar. This is because all native mammal groups that exist on Madagascar are believed to have colonized the island via overwater dispersal, which would account for this asymmetry (Simpson, 1940; Poux et al. 2005). Only *S. madagascariensis* has ever been suspected to be native to the island. *Suncus murinus,* on the other hand, is broadly distributed across the Old World and widely believed to have been introduced to Madagascar (Hutterer and Tranier, 1990). If native,

S. madagascariensis colonization is remarkable, since overwater dispersal is rare in shrews (Churchfield 1990).

The relationships among African shrews are far from resolved. Meester (1953) and Heim de Balsac and Lamotte (1956, 1957) suggested two major lineages of African shrews, but only one African shrew lineage remained recognized in the taxonomy. This division has been supported by recent molecular studies (Quérouil et al., 2001; Maddalena and Bronner, 1992). Originally a single subfamily, Crocidurinae has been divided into two subfamilies to include Myosoricinae (Hutterer, 2005).

Myosoricinae includes the genera *Congosorex* (3 species) (Stanley et al., 2005), *Surdisorex* (2 species) and *Myosorex* (13 species) (Hutterer, 2005). Crocidurinae includes the genera *Suncus* (18 species), *Sylvisorex* (17 species), *Ruwenzorisorex* (1 species), *Scutisorex* (1 species), *Crocidura* (179 species) and *Paracrocidura* (3 species) (Hutterer, 2005). This classification is largely in agreement with the morphological analysis by Heim de Balsac and Lamotte (1956; 1957). Their use of external, cranial and dental characters yielded two lineages among African shrews. More recently, Quérouil et al. (2001), using molecular methods, also detected two lineages. Most discrepancies between the morphological and phylogenetic data occur at the intrageneric level.

Mitochondrial DNA is widely used to infer phylogenetic relationships among mammalian species. Recently, mtDNA has been used to infer phylogenetic relationships among shrews in *Sylvisorex* (Stanley and Olson., 2005), *Crocidura* (Ruedi, 1998; Ruedi et al., 1998), *Sorex* (Fumagalli et al., 1999; Ohdachi et al., 2001; Ohdachi et al., 2006; Ohdachi et al., 2003), African shrews (Quérouil et al., 2001), and Soricidae as a whole (Fumagalli et al., 1999). Therefore,

using mtDNA seems appropriate to continue investigating the relationships within African shrews.

The first objective of this study is to test the taxonomic validity and phylogenetic position of *Suncus madagascariensis,* which is the only soricid purported to be native to Madagascar. Coquerel (1848) first described this species as *Sorex madagascariensis*. He cited such characteristics as a more slender tail, smaller incisors, a salient crown of the canine teeth and molars, and a smaller skull than *Suncus etruscus* (Coquerel, 1848). Subsequently, the status of *Suncus madagascariensis* was changed to a subspecies of the Old World *S. etruscus,* based solely on morphology (Heim de Balsac and Meester, 1977) Recently, *S. madagascariensis* was again elevated to full species status (Hutterer, 1993) The elevation of this species from *Suncus etruscus madagascariensis* to *Suncus madagascariensis* is highly speculative and *S. madagascariensis* is still often treated as a subspecies of *S. etruscus* (see Hutterer, 2005). Comparing *S. madagascariensis* to *S. etruscus* will give some insight as to *S. madagascariensis* status on Madagascar and its relationship to other crocidurine shrews. If *Suncus madagascariensis* is indeed native, it would represent the seventh documented natural colonization of Madagascar by terrestrial mammals (nesomyine rodents, tenrecs, lemuriform primates, carnivorans, extinct pygmy hippos, and *Plesiorycteropus*).

The other shrew species found on Madagascar, *Suncus murinus,* is known to be a human commensal and is presumably introduced (Hutterer and Tranier, 1990). Recently *S. murinus* was found harboring diseases commonly found in rodents, including plague and a strain of hantavirus (Duplantier et al., 2003; Klempa et al., 2007). If *S. murinus* is indeed transmitting diseases to humans, understanding its origin will be extremely valuable to researchers trying to track the

disease's movements and consequences. I sequenced two new samples of *S. murinus* in addition to the sequence from Quérouil et al. (2001) to gain some insight into the origin of *S. murinus* living on Madagascar.

I have chosen to use a portion of the mtDNA gene encoding the ribosomal subunit 16S, which was also used by Quérouil et al. (2001). The reasons for choosing 16S were largely practical. A large data set already existed on GenBank for African shrews from two previous studies (Quérouil et al., 2001; Quérouil et al., 2003). This allowed me access to a large number of samples for which fresh tissues are difficult to obtain. These sequences were added to new samples, resulting in multiple representatives of many species. Divergence within these species can be compared to understand one of the primary objectives in this study, which is the taxonomic designation of the only purported endemic shrew on Madagascar, *S. madagascariensis*.

One prior study attempted to resolve phylogenetic relationships among African shrews (Quérouil et al., 2001), but many relationships remain unresolved or are poorly supported. Using an updated alignment with reference to the secondary structure model of the mammalian 16S molecule from Burk et al. (2002), I reanalyzed the sequence data from Quérouil et al. (2001) with several additional species to further explore the phylogenetic relationships among African shrews.

Methods

DNA was isolated from frozen or buffered muscle or kidney tissues using the PureGene kit (Gentra Systems, Inc.) with reference to the animal tissues protocol. Primers used to amplify the 3' region of the 16S gene were 16S-F1 (5'-GTAAAAGGAACTCGGCAAACA-3') and 16S-

R1 (5' CCGGTCTGAACTCAGATCACGTA 3'). These span positions 1948-2489 of the *Crocidura russula* mitochondrial genome (GenBank accession AY769264).

PCR amplifications were performed in 25 µl reactions, including 5 µl unquantified DNA in a 1:50 dilution from the original extraction, 1 µl of each 10 mM primer, 2.5 µl 10x buffer with 1.5 mM $MgCl_2$, 0.25 µl of 5 U/µl Taq polymerase Promega, Madison WI, USA, and 0.5 µl dNTPs 100 mM. Additional $MgCl_2$ and water were added to bring the total concentration to 2.5 mM. All extractions and PCRs included negative controls. Thermal cycling parameters included two minutes at 94 °C followed by 30 cycles of 20 seconds at 94°C, 15 seconds at 55 °C, and 60 seconds at 72 °C, with a final two-minute extension at 72 °C. PCR products were purified using 1.25 µl Exo-SAP It (USB Inc.) per 5 µl of PCR reaction following manufacturer's instructions. Unquantified aliquots of the purified PCR products were cycle-sequenced for both strands using the amplification primers and 1 µl purified DNA, 0.5 µl BigDye Terminator v. 3.1 (Perkin-Elmer, Boston, MA, USA), 1.75 5X buffer, and water to make a total volume of 10 µl per reaction. Sequencing reactions were purified using Sephadex (MP Biomedicals, Inc.) according to the manufacturer's instructions. Samples were electrophoresed on an ABI 3100 automated sequencer (Perkin-Elmer). Resulting sequences were imported and edited in Sequencher 4.2 (Genecodes, Ann Arbor, MI). The resulting sequences have been deposited in GenBank (accessions numbers EF507185-EF507209).

A total of 59 sequences was analyzed, 34 of which were taken from GenBank, with the remaining 25 generated in this study. Twelve species were not included in the Quérouil et al. (2001) study (Table 1). All African genera of shrews, except *Surdisorex,* were represented. *Sorex araneus* (AF274562) and *Soriculus fumidus* (AF274561) (Soricinae) were used as

outgroups. Quérouil et al. (2001) showed that the African shrews comprise a monophyletic clade, making Soricinae an appropriate outgroup.

Sequence alignment. Sequences were aligned by eye with reference to the secondary structure model for mammalian 16S (Burk et al., 2002). Regions that could not be confidently aligned were excluded from all analyses. These included missing flanking ends (1-912 and 1470-1665 of the aligned 16S gene) and unpaired regions of the secondary structure located at positions 1154-1206, 1258-1258, 1292-1317 and 1321-1327 of the alignment. The resulting alignment was 531 bp long. The alignment is shown in Appendix 1.

Phylogenetic analyses. Phylogenetic analyses were performed under the maximum parsimony (MP) and maximum likelihood criteria (ML) using PAUP* 4.0b (Swofford, 2003). For MP tree searches all characters were treated as unordered and equally weighted. Heuristic tree searches were conducted using stepwise addition (100 random addition sequences) and the TBR branch-swapping algorithm. Parsimony bootstrap support values were obtained with 100 random addition sequences and 1000 pseusdoreplicates with the TBR branch-swapping algorithm. For ML tree searches, a single neighbor-joining tree was used to estimate the parameters of nucleotide substitution using ModelTest 3.0 (Posada and Crandall, 1998; 2001). Parameter values were then fixed in a search employing 100 heuristic replicates with TBR branch swapping. ML Bootstrap support was obtained from 1000 pseusdoreplicates with the SPR branch-swapping algorithm.

Bayesian posterior probabilities were estimated to assess nodal support using MrBayes (Ronquist and Hueslenbeck, 2005). Covarying nucleotides that form pair bonds in transcribed RNA molecules do not evolve independently of each other and therefore violate the assumption

of character independence in phylogenetic analysis (Dixon and Hillis, 1993). I have taken advantage of the feature in MrBayes that allows for the incorporation of a user-specified model of secondary structure. Analyses were conducted both including and excluding information regarding canonical pairs found in the secondary structure model of Burk et al. (2002). This was done to see if the resulting topologies and/or support values differed depending on the model. For both analyses, four MCMC chains (three heated, one unheated) were allowed to proceed for 10 million generations, sampling trees every 100 generations. Burn-in was where likelihood values were highly variable, and therefore discarded. All remaining trees were used to estimate posterior probabilities.

Results

Sequence characteristics. Uncorrected pairwise distances between species (ingroup only) ranged from 1.58% - 18.7%. Intraspecific distances ranged from 0.1% - 3.04% in the nine species for which there were two or more individuals sequenced with nonidentical haplotypes (Table 2).

Phylogenetics. MP heuristic searches resulted in 24 equally parsimonious trees of 1311 steps. A strict consensus of these with bootstrap support is shown in Figure 1. *Suncus madagascariensis* and *S. etruscus* were recovered as sister species with 93% bootstrap support. *Sylvisorex granti* was found to nest within *S. ollula* but with only 63% bootstrap support. A clade uniting *Congosorex* and *Myosorex* was recovered with 87% bootstrap support, with *Congosorex* recovered as paraphyletic. *Congosorex phillipsorum* was sister to a clade uniting *Congosorex* sp., *Myosorex geata,* and *M. kihaulei* with 96% support. *Suncus* and *Sylvisorex* were

not supported as monophyletic genera, which conforms to previous studies (Quérouil et al., 2001; Stanley and Olson, 2005).

The preferred model of nucleotide substitution was GTR with a gamma distribution (shape parameter = 0.231), the proportion of invariant sites equal to 0.453 and the rate matrix values were 5.201, 10.110, 9.954, 0.240, 40.923. The best tree obtained in the heuristic ML analysis had a likelihood score of -ln= 2864.12. Bootstrap support values are shown in Figure 1. ML Bootstrap support values were very similar to MP bootstrap scores (Figure 1)

Likelihood scores converged on a stable value before 175,000 generations in the Bayesian analysis. The first 25,000 samples were therefore excluded as burn-in. Resulting posterior probabilities for both Bayesian analyses are shown in Figure 1. Most relationships recovered with high MP and ML bootstrap support were also recovered with high support in the Bayesian analysis. Conflicting results between bootstrap values (MP and ML) and posterior probabilities occurred where nodes had minimal to low support. A clade consisting of *Crocidura* and *Paracrocidura* was recovered with a posterior probability of 0.96, which is noteworthy due to support of less than 50% in the MP bootstrap analyses. The clade consisting of *Sylvisorex ollula* and *S. granti* received much higher support in the Bayesian analysis than in the bootstrap analyses, with 0.99 posterior probabilities. Incorporation of a secondary structure produced lower posterior probabilities, compared to analysis without. Values were often comparable to both MP and ML bootstrap values (Figure 1).

Discussion

Alignments. This study relied heavily on an alignment based on the secondary structure model for the mammalian 16S rRNA molecule (Burk et al, 2002). The secondary structure of

this molecule consists of loops (non-pairing regions) and stems (pairing regions). Insertion/deletion events (indels), which are found primarily in loops, can be difficult to align due to higher rates of evolution than in the pairing segments (Springer and Douzery, 1996; Springer et al., 1995; Vawter and Brown, 1993). Regions with alignment ambiguity can, when included in analyses, result in incorrect topologies. This study used an updated 16S model (Burk et al., 2002) from the model (De Rijk, 1995) used originally by Quérouil et al. (2001). The biggest difference in this alignment and that of Quérouil et al. (2001) is in the number of excluded positions. I tended to err on the side of caution, and as a result I excluded 93 sites in this analysis due to ambiguity. By excluding so many sites, I may have omitted potentially informative variation. Although the result may be lower support values and more polytomies, more conservative choices in the exclusion of alignment-ambiguous positions reduces the possibly of inferring the wrong phylogeny.

Few studies have incorporated secondary structure by way of the doublet model in MrBayes. Olson et al. (2005) found minimal differences in posterior probabilities in analyses of the mitochondrial 12S rRNA gene in tree shrews with and without the incorporation of secondary structure. One of the other studies to carry out such a comparison was of insect phylogeny using the 18S rRNA gene (Kjer, 2004). Although some nodes did exhibit differences in posterior probabilities between the two analyses, neither model gave consistently lower or higher posterior probabilities than the other.

African shrews. Results support the monophyly within of the two subfamilies of African shrews, which was also recovered by Quérouil et al. (2001). These two subfamilies have also been supported by behavior (Meester, 1953; Heim de Balsac and Lamotte, 1957) and anatomical

(Butler, 1998) features. However, there are many discrepancies within several genera within these subfamilies that conflict with current taxonomy.

For Myosoricinae these results suggest the genus *Congosorex* is paraphyletic (Figure 1). *Congosorex* sp. consistently grouped with *Myosorex geata* and *M. kihaulei,* with *C. phillipsorum* basal to this clade. *Congosorex phillipsorum* is the first of this genus to be discovered in the Udzungwa forest, Tanzania (Stanley et al., 2005). All other species in this genus occur within the central forest block of the Republic of Congo and the Central African Republic (Stanley et al., 2005). Homewood and Rodgers (1981) and Dinesen et al. (1994) that the Udzungwa forest is a refugium for historic populations of ancestral lineages. This may explain why we are seeing a split between *C. phillipsorum* from the other *Congosorex* species.

There is no strong support for the monophyly of several genera within Crocidurinae, in which there was no support value above 50%. One exception was *Crocidura*, which exhibited high posterior probabilities but had no support above 50% for either bootstrap analysis. Polyphyly may suggest a rapid speciation early in the history of shrews (Heim de Balsac and Meester, 1977). An analysis incorporating more of the mitochondrial and nuclear genomes is clearly needed.

In my analyses, *Paracrocidura* was nested within the genus *Crocidura.* The clade including both genera is well supported in this study. This suggests that *Paracrocidura* evolved recently and shows some genetic (Quérouil et al., 2001) and morphological (Hutterer, 1986) differences from *Crocidura.*

Suncus and *Sylvisorex* were displayed as paraphyletic genera, which has been suggested previously (Butler, 1998; Butler and Greenwood, 1979; Hutterer, 2005; Quérouil et al., 2001;

Stanley and Olson, 2005). With *Suncus* and *Sylvisorex* relationships poorly supported, it is difficult for me draw any firm conclusions regarding suggested taxonomic changes.

Suncus lixus and *S. varilla* are nested within a clade also containing *Sylvisorex vulcanorum* and *Sylvisorex howelli,* none of which was included in Quérouil et al. (2001). This is the first mtDNA study to include *S. varilla* and *S. lixus.* The more noteworthy finding was the moderate support for the basal position of *S. varilla* with respect to *Sylvisorex howelli.* Stanley and Olson (2005) found no strong evidence as to the closest relative of *S. howelli.*

My results suggested different relationships than those of Quérouil et al. (2001) for the two monotypic genera of African shrews, *Ruwenzorisorex* and *Scutisorex. Ruwenzorisorex* appeared to be most closely related to *Sylvisorex cf. konganensis*, but with questionable support. The other result that differed from the Quérouil et al. (2001) study was the position of *Scutisorex somereni*. It was found to be basal to a clade containing *Sylvisorex ollula* and *S. granti.* However, this relationship was poorly supported. *S. ollula* was grouped by Quérouil et al. (2001) with *Sylvisorex cf. konganensis;* my analysis did not recover any relationship of *S. ollula* with *Sylvisorex cf. konganensis.* Furthermore, Stanley and Olson (2005) found that *S. ollula* was more closely related to *S. megalura*, although their data set utilized different mitochondrial genes than the present study.

Madagascar's shrews. As to the endemic status of *Suncus madagascariensis,* my results suggest that *S. madagascariensis* is not distinct from *S. etruscus* and was likely introduced. There is only 1.58% (uncorrected) divergence between the *S. etruscus* specimen sequenced by Quérouil et al. (2001) (collected in France) and the *S. madagascariensis* specimen sequenced in this study. This is well within the range observed in other crocidurine species (Table 2). Four of

the species showed higher intraspecific divergence over much smaller geographic distances (*Sylvisorex howelli, S. ollula, S. johnstoni,* and *S. megalura*). The holotype of *Suncus madagascariensis* has been lost (Goodman et al., 1999), but measurements suggest that it is very similar to that of *S. etruscus.* Heim de Balsac and Meester (1977) tentatively placed it in *S. etruscus* but Hutterer (1993) elevated it to full species status. However, whether this species was introduced or native is still unclear. An analysis of NADH dehydrogenase subunit 2 (ND2) of 30 specimens of *S. madagascariensis* from across the island showed little to no differentiation (see Chapter 1), suggesting that it is a recent addition to Madagascar's mammal fauna.

Suncus murinus, the only other shrew on Madagascar, was introduced (Hutterer and Tranier, 1990) and is a known human commensal (Hutterer and Tranier, 1990). My results suggest minimal divergence within *S. murinus* from Madagascar, the Philippines and Indonesia, in fact, only 0.5%, much less than between *S. madagascariensis* and *S. etruscus.* Gene flow may also be contributing to the lack of variation across in my samples. Hutterer and Tranier (1990) suggested that *Suncus murinus* was introduced to Madagascar via trading routes between the 11th and 14th centuries. This introduction was most likely via shipping routes between Arabia, the Red Sea, or Africa (Hutterer and Tranier, 1990). However, my study lacked samples from these locations. With the samples I used in this study the relationships between *S. murinus* on Madagascar, the Philippines and Indonesia are unresolved due to the lack of variability in my samples. Better understanding of the origin of Madagascar's populations of *S. murinus* requires samples from throughout its entire range, as well as more rapidly evolving markers than the one used in this study.

These results suggest that more extensive studies are needed for Malagasy shrews. Relationships between different continental populations of both species' known ranges are not well understood. In general, my results are largely in agreement with those of Quérouil et al. (2001) with respect to the monophyly of the two subfamilies within the African shrews. Strongly supported relationships were similar to those of Quérouil et al. (2001). The most insight of phylogenetic relationships was gained by the inclusion of more samples to the original data set as well as more extensive analyses. The use of an updated alignment does not appear to be a major contributing factor to discrepancies between this study and Quérouil et al. (2001). Discrepancies can most likely be attributed to the addition of more samples and additional analyses. This study contributed more information that was lacking in Quérouil et al. (2001). More can be learned about the relationships of African shrews with additional samples and genes.

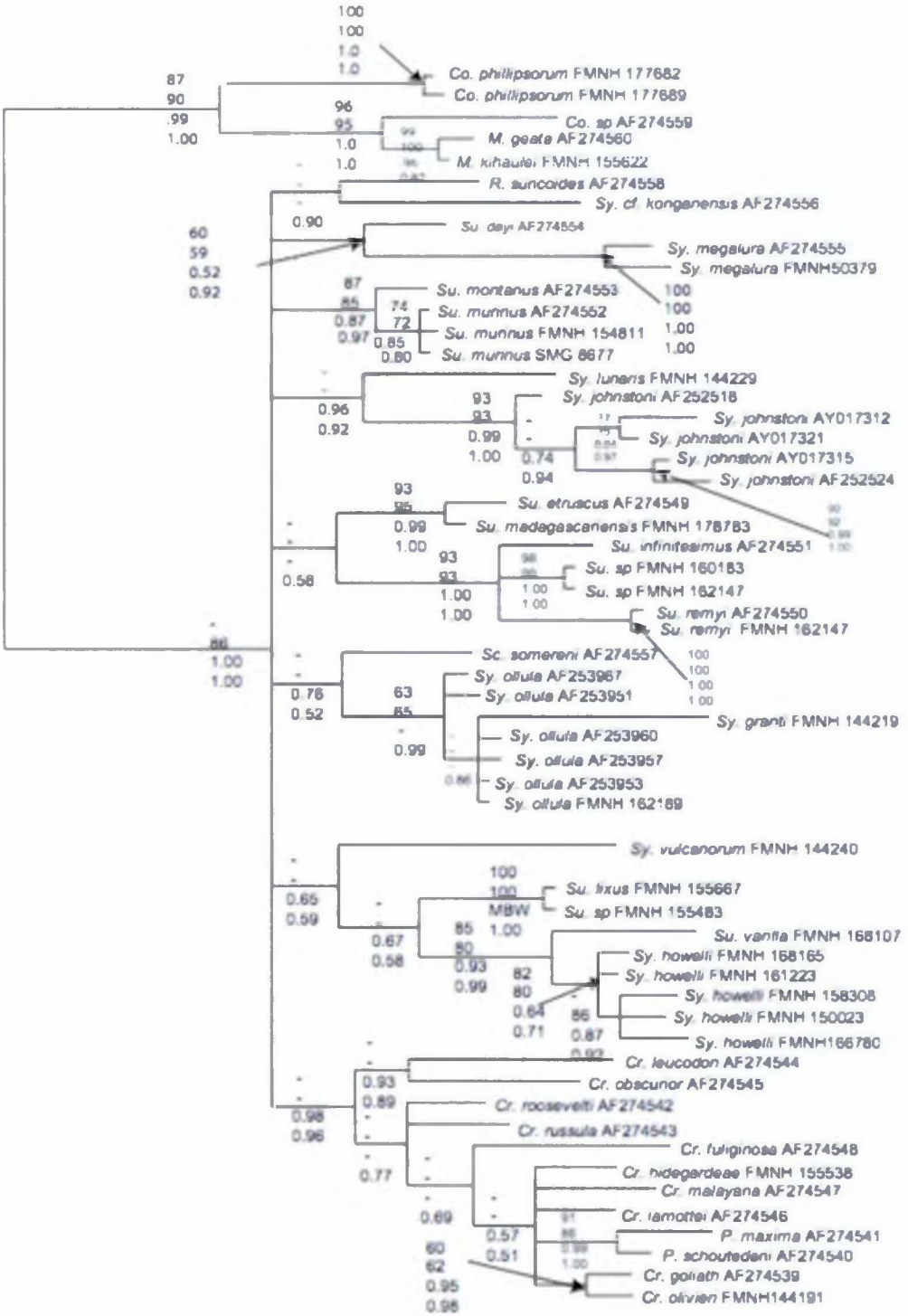
Table 2.1 Specimens analyzed in this study are from the Field Museum of Natural History (FMNH) and numbers represent their FMNH catalogue numbers. One specimen not noted with a FMNH number was collected by Steven M. Goodman (SMG) and a field number follows initials.

Museum	Catalog Number	Genus	Species	Country
FMNH	177682 (holotype)	*Congosorex*	*phillipsorum*	FMNH
FMNH	177689	*Congosorex*	*phillipsorum*	FMNH
FMNH	155538	*Crocidura*	*hildegardeae*	FMNH
FMNH	144191	*Crocidura*	*olivieri*	FMNH
FMNH	155622	*Myosorex*	*geata*	FMNH
FMNH	155667	Suncus	lixus	FMNH
FMNH	162147	*Suncus*	*remyi*	Gabon
FMNH	155483	*Suncus*	*sp.*	Tanzania
FMNH	160183	*Suncus*	*sp.*	Uganda
FMNH	160184	*Suncus*	*sp.*	Uganda
FMNH	168107	*Suncus*	*varilla*	Tanzania
FMNH	168108	*Suncus*	*varilla*	Tanzania
FMNH	178783	*Suncus*	*madagascariensis*	Madagascar
FMNH	144219	*Sylvisorex*	*granti*	
FMNH	150023	*Sylvisorex*	*howelli*	Tanzania
FMNH	168165	*Sylvisorex*	*howelli*	Tanzania
FMNH	158308	*Sylvisorex*	*howelli*	Tanzania
FMNH	166780	*Sylvisorex*	*howelli*	Tanzania
FMNH	161223	*Sylvisorex*	*howelli*	Tanzania
FMNH	150011	*Sylvisorex*	*howelli*	Tanzania
FMNH	162197	*Sylvisorex*	*johnstoni*	
FMNH	144229	*Sylvisorex*	*lunaris*	
FMNH	150379	*Sylvisorex*	*megalura*	
FMNH	162189	*Sylvisorex*	*ollula*	
FMNH	144237	*Sylvisorex*	*vulcanorum*	
FMNH	144240	*Sylvisorex*	*vulcanorum*	
FMNH	144240	*Sylvisorex*	*vulcanorum*	
FMNH	154811	*Suncus*	*murinus*	Philippine islands
SMG	8677	*Suncus*	*murinus*	Madagascar

Table 2.2: Intraspecific pairwise distances (% uncorrected)

Species	Number of Specimens	Pairwise distance (uncorrected)
Sylvisorex howelli	5	0.9 – 3.04
Sylvisorex ollula	6	0.3 – 1.9
Sylvisorex ollula + S. granti	7	0.3 – 3.8
Congosorex phillipsorum	2	0.3
Suncus murinus	3	0.1 – 0.5
Sylvisorex johnstoni	5	1.1 – 8.5
Sylvisorex megalura	2	3.2
Suncus remyi	2	0.19
Suncus etruscus + S. madagascariensis	2	1.58

Figure 2.1: Maximum likelihood tree with bootstrap values and posterior probabilities (outgroup not shown). First and second numbers represent maximum parsimony and maximum likelihood bootstrap support values (respectively) after 1000 pseudoreplicates. Bayesian posterior probabilities are reported with and without the incorporation of secondary structure (third and fourth numbers respectively). Bootstrap values < 50% are represented with a – , as are posterior probabilities less than .5. Abbreviations of genus names are as follows: *Congosorex* (*Co.*), *Myosorex* (*M.*), *Sylvisorex* (*Sy.*), *Suncus* (*Su.*), *Ruwenzorisorex* (*R.*), *Scutisorex* (*Sc*), *Crocidura* (*Cr.*), *Paracrocidura* (*P.*). Numbers after the species names are FMNH catalogue numbers. Steven M. Goodman (SMG) collected the one specimen not noted with a FMNH number and number shown is a field number.


Co. philipsorum FMNH 177682
Co. philipsorum FMNH 177689
Co. sp AF274559
M. geata AF274560
M. kihaulei FMNH 155622
R. suncoides AF274558
Sy. cf. kongonensis AF274556
Su. dayi AF274554
Sy. megalura AF274555
Sy. megalura FMNH50379
Su. montanus AF274553
Su. murinus AF274552
Su. murinus FMNH 154811
Su. murinus SMG 8677
Sy. lunaris FMNH 144229
Sy. johnstoni AF252518
Sy. johnstoni AY017312
Sy. johnstoni AY017321
Sy. johnstoni AY017315
Sy. johnstoni AF252524
Su. etruscus AF274549
Su. madagascariensis FMNH 178783
Su. infinitesimus AF274551
Su. sp FMNH 160183
Su. sp FMNH 162147
Su. remyi AF274550
Su. remyi FMNH 162147
Sc. somereni AF274557
Sy. ollula AF253967
Sy. ollula AF253951
Sy. granti FMNH 144219
Sy. ollula AF253960
Sy. ollula AF253957
Sy. ollula AF253953
Sy. ollula FMNH 162189
Sy. vulcanorum FMNH 144240
Su. lixus FMNH 155667
Su. sp FMNH 155483
Su. vanita FMNH 168107
Sy. howelli FMNH 168165
Sy. howelli FMNH 161223
Sy. howelli FMNH 158308
Sy. howelli FMNH 150023
Sy. howelli FMNH166780
Cr. leucodon AF274544
Cr. obscurior AF274545
Cr. roosevelti AF274542
Cr. russula AF274543
Cr. fuliginosa AF274548
Cr. hidegardeae FMNH 155538
Cr. malayana AF274547
Cr. lamottei AF274546
P. maxima AF274541
P. schoutedeni AF274540
Cr. goliath AF274539
Cr. olivieri FMNH144191

References

Burk, A., Douzery, E.J.P., Springer, M.S., 2002. The secondary structure of mammalian mitochondrial 16S rRNA molecules: Refinements based on a comparative phylogenetic approach. Jour. Mamm. Evol. 9, 225-252.

Butler, P.M., 1998. Fossil history of shrews in Africa. Evolution of shrews. Mammal Research Institute, Bialowieza, pp. 121-132.

Butler, P.M., Greenwood, M., 1979. Soricidae (Mammalia) from the Early Pleistocene of Olduvaï Gorge, Tanzania. Zool. Jour. Linn. Soc. 67, 329-379.

Churchfield, S., 1990. The natural history of shrews, A & C Black, London.

Coquerel, M.C., 1848. Súr une espèce nouvelle de musaraigne trouvée a Madagascar. Annales des Sciences Naturelles 1, 195-198.

De Rijk, P., 1995. Optimizations of a database for ribosomal RNA structure and application in structural and evolutionary research. Ph. D. dissertation, University of Antwerp, Antwerp.

Dinesen, L., Lehmberg, T., Svendsen, J.O., Hansen, L.A., Fjeldsa, J., 1994. A new genus and species of perdicine bird (Phasianidae, Perdicini) from Tanzania - a Relict Form with Indo-Malayan Affinities. Ibis 136, 3-11.

Dixon, M.T., Hillis, D.M., 1993. Ribosomal RNA secondary structure; compensatory mutation and implications for phylogenetic analysis. Mol. Biol. Evol. 10, 256-267.

Duplantier, J.M., Catalan, J., Orth, A., Grolleau, B., Britton-Davidian, J., 2003. Systematics of the black rat in Madagascar: consequences for the transmission and distribution of plague. Biological Journal of the Linnean Society 78, 335-341.

Goodman, S.M., Jenkins, P.D., Pidgeon, M., 1999. Lipotyphla (Tenrecidae and Soricidae) of the Reserve Naturelle Integrale d' Andohahela, Madagascar. In: Goodman, S.M. (Ed.), A floral and faunal inventory of the Reserve Naturelle Intefrale D'Andohahela, Madagascar: with reference to elevational variation: zoology, New series. Field Museum of Natural History, Chicago. Vol 11. 189-216

Fumagalli, L., Taberlet, P., Stewert, D.T., Gielly, L., Hausser, J.,Vogel, P., 1999. Molecular phylogeny and evolution of *Sorex* shrews (Soricidae: Insectivora) inferred from mitochondrial DNA sequence data. Mol. Phylogentic. Evol. 11: 222-235

Heim de Balsac, H., Lamotte, M., 1956. Evolution et phylogenie de Soricides africains-I. Mammalia 20, 140-167.

Heim de Balsac, H., Lamotte, M., 1957. Evolution et phylogenie de Soricides africains-II. Mammalia 21, 16-46.

Heim de Balsac, H., Meester, J., 1977. Order Insectivora. In: Messter, J. and Setzer, H. W. (Eds.), The mammals of Africa: an identification manual. Smithsonian Institute, Washington D.C. 1-29

Homewood, K.M., Rodgers, W.A., 1981. A previously undescribed mangabey from southern Tanzania. Int. J. Primatol. 2, 47-55.

Hutterer, R., 1993. Order Insectivora. In: Wilson, D.E., and D. M. Reeder (Ed.), Mammal species of the world: A taxonomic and geographic reference. Smithsonian Institution Press, Washington D.C. 69-130.

Hutterer, R., 2005. Order Soricomorpha. In: Wilson, D.E., and D. M. Reeder (Ed.), Mammal species of the world: a taxonomic and geographic reference. Johns Hopkins University Press. Baltimore. Vol. 1: 220-260.

Hutterer, R., Tranier, M., 1990. The immigration of the Asian house shrew (*Suncus murinus*) into Africa and Madagascar. In: Peters, G., Hutterer, R. (Eds.), Vertebrates of the tropics. Museum Alexander Koenig, Bonn. 309-319

Hutterer, R., 1986. Synopsis der Gattung *Paracrocidura* (Mammalia: Soricidae), mit Beschreibung einer neuen Art. Bonn. Zool. Beiter. 37, 73-90.

Kjer, K.M., 2004. Aligned 18S and insect phylogeny. Syst. Biol. 53, 506-514.

Klempa, B., Fichet-Calvet, E., Lecompte, E., Auste, B., Aniskin, V., Meisel, H., Barrière, P., Koivogui, L., Meulen, J., Krüger, D.H., 2007. Novel hantavirus sequences in shrew, Guinea. Emerging Infectious Diseases 13, 520-522.

Maddalena, T., Bronner, G., 1992. Biochemical systematics of the endemic African genus *Myosorex* gray, 1838 (Mammalia, Soricidae). Israel Jour. of Zool. 38, 245-252.

Meester, J., 1953. The genera of African shrews. Ann. Transvaal Mus. 22, 205-214.

Ohdachi, S., Dokuchaev, N.E., Hasegawa, M., Masuda, R., 2001. Intraspecific phylogeny and geographical variation of six species of northeastern Asiatic *Sorex* shrews based on the mitochondrial cytochrome b sequences. Mol. Ecol. 10, 2199-2213.

Ohdachi, S., Hasegawa, M., Iwasa, M.A., Vogel, P., Oshida, T., Lin, L.-K., Abe, H., 2006. Molecular phylogenetics of soricid shrews (Mammalia) based on mitochondrial cytochrome b gene sequences: with special reference to Soricinae. Jour. Zool. 270, 177-191.

Ohdachi, S.D., Abe, H., Han, S.H., 2003. Phylogenetical positions of *Sorex* sp. (Insectivora, Mammalia) from Cheju Island and *S. caecutiens* from the Korean Peninsula, inferred from mitochondrial cytochrome b gene sequences. Zool. Sci 20, 91-95.

Olson, L.E., Sargis, E.J., Martin, R.D., 2005. Intraordinal phylogenetics of treeshrews (Mammalia: Scandentia) based on evidence from the mitochondrial 12S rRNA gene. Mol. Phylogenet. Evol. 35, 656-673.

Posada, D., Crandall, K.A., 1998. ModelTest: testing the model of DNA substitution. Bioinformatics 14, 817-818.

Posada, D., Crandall, K.A., 2001. Selecting the best-fit model of nucleotide substitution. Syst Biol 50, 580-601.

Poux, C., Madsen, O., Marquard, E., Vieites, D.R., de Jong, W.W., Vences, M., 2005. Asynchronous colonization of Madagascar by the four endemic clades of primates, tenrecs, carnivores, and rodents as inferred from nuclear genes. Syst Biol 54, 719-730.

Quérouil, S., Hutterer, R., Barriere, P., Colyn, M., Kerbis Peterhans, J.C., Verheyen, E., 2001. Phylogeny and evolution of African shrews (Mammalia: Soricidae) inferred from 16S rRNA sequences. Mol. Phylogenet. Evol. 20, 185-195.

Quérouil, S., Verheyen, E., Dillen, M., Colyn, M., 2003. Patterns of diversification in two African forest shrews: *Sylvisorex johnstoni* and *Sylvisorex ollula* (Soricidae, Insectivora) in relation to paleo-environmental changes. Mol. Phylogenet. Evol. 28, 24-37.

Ronquist, F., Hueslenbeck, J.P., 2005. MrBayes: Phylogenetic inference under mixed models, 3.1.1., Uppsala, Sweden.

Ruedi, M., 1998. Protein evolution of shrews. Mammal Research Institute, Polish Academy of Science, Bialowieza.

Ruedi, M., Auberson, M., Savolainen, V., 1998. Biogeography of Sulawesian shrews: testing for their origin with a parametric bootstrap on molecular data. Mol. Phylogenetic Evolution 9, 567-571.

Simpson, G.G., 1940. Mammals and landbridges. J. Washington Acad. Sci. 30, 137-163.

Springer, M.S., Douzery, E., 1996. Secondary structure and patterns of evolution among mammalian mitochondrial 12S rRNA molecules. J. Mol. Evol. 43, 357-373.

Springer, M.S., Hollar, L.J., Burk, A., 1995. Compensatory substitutions and the evolution of the mitochondrial 12S rRNA gene in mammals. Mol Biol Evol 12, 1138-1150.

Stanley, W.T., Olson, L.E., 2005. Phylogeny, phylogeography, and geographic variation of *Sylvisorex howelli*, an endemic shrew of the Eastern Arc Mountains. J. of Zool. 266, 341-354

Stanley, W.T., Rogers, M.A., Hutterer, R., 2005. A new species of *Congosorex* from the Eastern Arc Mountains, Tanzania, with significant biogeographical implications. J. Zool. 265, 269-280.

Swofford, D.L., 2003. PAUP*: Phylogenetic analysis using parsimony (and other methods). Sinauer Associates, Sunderland, MA.

Vawter, L., Brown, W.M., 1993. Rates and patterns of base change in the small subunit ribosomal-RNA gene. Genetics 134, 597-608.

Appendix 1.1

Sequences from GenBank

Sylvisorex howelli **AY691831**, **AY691830**, **AY691829**, **AY691828**, **AY691827**, **AY691826**, *Sylvisorex johnstoni* **AY691832**, *Sylvisorex lunaris* **AY691833**, *Sylvisorex megalura* **AY691834**, *Sylvisorex granti* **AY691835**, *Sylvisorex vulcanorum* **AY691836**, *Sylvisorex ollula* **AY691837**, *Crocidura olivieri* WTS 459, *Crocidura hildegardeae* **AY691821**, *Suncus murinus* **AY691841**

Appendix 1.2

Specimens analyzed

All samples were collected on Madagascar unless stated otherwise. Museum catalog numbers are listed under the species followed by their respective locality (grouped by province). All catalog numbers are FMNH (=Field Museum of Natural History) unless otherwise indicated. "SMG" refers to as-yet uncatalogued specimens collected by S.M. Goodman. "AMNH RAX" refers to specimens collected by C. J. Raxworthy and deposited at the American Museum of Natural History. "UADBA" refers to specimens housed at the Département de Biologie Animale, Université d'Antananarivo.

Suncus madagascariensis:

Toliara:

151949, 151950: Forêt de Zombitsy 22°51'S 44°43'E, 870 m; 156493, 156605: Réserve Naturelle Intégrale d'Andohahela, parcel 2, 7.5 km ENE Hazofotsy 24°49.0'S, 46°36.6'E, 120 m; 173145-172148: Parc National de Tsimanampetsotsa, 6.5 km NE Efoetse, in forest around Mitoho Cave, 24°03.0'S, 43°45.0'E, 50 m; 173149, 173150: Parc National de Tsimanampetsotsa, 21.5 km NE Efoetse, along old petroleum prospection road, 24°00.5'S, 43°53.9'E, 100 m; 173241: Anafiky, 23° 19' 16.0"S, 44° 04' 39.1" E, 50m; 176238: Forêt de Mikea, 8.4 km SSE of Befandefa, 22° 13.0' S, 63° 19.8' E, 50 m.

Mahajanga:

167543: Réserve Spéciale d'Ambohijanahary, Foret d'Ankazotsihitafototra, 18°15.7'S, 45°25.2'E, 1150m; 178553, 178554: RNI de Namoroka, Site Andiziabe, 2.0 km SE Namoroka (village), 16°

24.4'S, 45° 18.4'E, 110 m; 177297, 177298, 177325, SMG 13636: Station Forestier d'Ampijoroa, Jardin Botanique A, 16° 19.4'S, 46° 48.4'E, 100 m.

Antsiranana:

AMNH RAX 4171: Forêt d'Analafiana, à l'E de la rivière Antsahambovo, 6 km N. d'Andilana, 13° 26.73' S, 49° 50.118' E, 50 m; 178783: Reserve Speciale d'Analamenana, 8.6 km SE Menagisy, Foret d'Analabe, along Bobalcrudizo River, 12° 45.0'S, 49° 29.6'E, 40 m; 178859: RS d'Analamenana, Foret d'Ankavanana, 15.8 km SE Anivorano-Nord, 12° 47.7'S, 49° 22.1'E, 200 m.

Suncus murinus:

Toliara:

176115: Parc National de Kirindy-Mite, 13 km W. Marofihitsa, 20° 47.4'S, 44° 8.8'E, 30 m.

Fianarantsoa:

UADBA SMG10634: Forêt d'Ianasana, 7 km W. Itremo, along Atsirakamhaity River, 20° 36.1'S, 46° 34.3'E, 1630 m.

Antsiranana:

154598: Parc National de la Montagne d'Ambre, near Station des Roussettes, 5.5 km SW Joffreville, 12° 31'38"S, 49° 10'18"E, 1000 m; 166242: Reserve Speciale de Manongarivo, 12.8 km (228°) SW Antanambao, 13° 58.6'S, 48° 25.4'E, 785 m; SMG 10781: Reserve Speciale de Manongarivo, 14.5 km (220°) SW Antanambao, 14° 0.0'S, 48° 25.7'E, 1240 m.

Toamasina:

165448: Forestière de Tampolo, 17° 17.2'S, 49° 24.5'E, 10 m; UADBA VHV042: Forêt de Vohitaly, 5.0 km SE Anjiahely, 15° 26.358' S, 49°, 32.062' E, 540-580 m; UADBA VHV114: Forêt de Plateau de Makira, Forêt d'Anmanaharibe, 3 km. E Soanafindra, 15° 11.407' S, 49° 36.866'E, 480-1150 m; 183968: Foultpointe, Forêt Andalava, 17° 42' 7.0"S, 49° 27' 31.1" E, 77m.

Antananarivo:

165499: Réserve Spéciale d'Ambohitantely, 24 km NE Ankazobe, 18° 10.1'S, 47° 16.6'E, 1450 m.

Philippine islands

154811-154813: Camiguin Is.; 167373: Mindanao Is.

Appendix 2.1

16S alignment

Data matrix and alignment of all 16S sequences used in all analyses. Species names are followed either by a GenBank Accession number, Abbreviations of genus names are as follows: *Congosorex* (*Co.*), *Myosorex* (*M.*), *Sylvisorex* (*Sy.*), *Suncus* (*Su.*), *Ruwenzorisorex* (*R.*), *Scutisorex* (*Sc*), *Crocidura* (*Cr.*), *Paracrocidura* (*P.*). Numbers after the species names are FMNH catalogue numbers. Steven M. Goodman (SMG) collected the one specimen not noted with a FMNH number and number shown is a field number.

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SPECIES                              880                                                                939
                                      |                                                                  |
So. araneus AF274562                 ??????????????????????????????CACCTCTAGCATTACTAGTATTAGAGGCACTGC
Sor. fumidus AF274561                ??????????????????????????????CACCTCTAGCATTTCTAGTATTAGAGGCACTGC
Co.sp AF274559                       ????????????????????????????????????AGCATTACAAGNATTAGAGGCACTGC
Co.phillipsorum FMNH 177632          ?AAACCCCGCCTGTTTACCAAAAACATCACCTCTAGCATTACAAGTATTAGAGGCACTGC
Co.phillipsorum FMNH 177689          AAAACCCCGCCTGTTTACCAAAAACATCACCTCTAGCATTACAAGTATTAGAGGCACTGC
My. geata AF274560                   ????????????????????????????????????CAGCACAACAAGTATTAGANGCANTGC
My. kihaulei FMNH 155622             TAAACCCCGCCTGTTTACCAAAAACATCACCTCTAGCATAACAAGTATTAGAGGCACTGC
Cr. fuliginosa AF274548              ????????????????????????????????????CTAGCATTATNNNTATTAGAGGCACTGC
Cr. goliath AF274539                 ????????????????????????????????????CTAGCATTACCAGTATTAGAGGCACTGC
Cr. hidegardeae FMNH 155538          CAAACCCCGCCTGTTTACCAAAAACATCACCTCTAGCATTATCAGTATTAGAGGCACTGC
Cr. lamottei AF274546                ??????????????????????????????CACNTCTAGCATTACCAGTATTAGAGGCACTGC
Cr. leucodon AF274544                ??????????????????????????????CCTCTAGCATTACTAGTATTAGAGGCACTGC
Cr. malayana AF274547                ??????????????????????????????ACATCTAGCATTACCAGTATTAGAGGCACTGC
Cr. obscurior AF274545               ??????????????????????????????CACCTCTAGCATTACCAGTATTAGNNGCACTGC
Cr. olivieri FMNH 144191             TAAACCCCGCCTGTTTACCAAAAACATCACCTCTAGCATTACCAGTATTAGAGGCACTGC
Cr. roosevelti AF274542              ????????????????????????????????????CCTTAGCATTACTAGTATTAGAGGCACTGC
Cr. russula AF274543                 ????????????????????????????????????CCTCTAGCATCATTAGTATTAGAGGCACTGC
P. maxima AF274541                   ??????????????????????????????CACCTCTAGCATTACTAGTATTAGAGGCATTGC
P. schoutedeni AF274540              ????????????????????????????????????TAGCATTACTAGTATTAAAGGCACTGC
R. suncoides AF274558                ??????????????????????????????CACCTCTAGCATTACCAGTATTAGAGGCACTGC
Sc. somereni AF274557                ????????????????????????????????????CTAGCATAATAAGTATTAGAGGCACTGC
Su. dayi AF274554                    ??????????????????????????????CACCTCTAGCATTACCAGTATTAGAGGCACTGC
Su. etruscus AF274549                ??????????????????????????????CACCTCTAGCATTACCAGTATTAGAGGCACTGC
Su. infinitesimus AF274551           ????????????????????????????????????CATNNCAAGTATTAGAGGCACTGC
Su. madagascariensis FMNH 178783     TAAACCCCGCCTGTTTACCAAAAACATCACCTCTAGCATTACCAGTATTAGAGGCACTGC
Su. montanus AF274553                ????????????????????????????????????TAGCATTTTCANNNTTAGAGGCACTGC
Su. murinus AF274552                 ????????????????????????????????????TAGCATTATCANNNTTAGAGGCACTGC
Su. murinus SMG 8677                 ??????????????????????AAACATCACCTCTAGCATTATCAGTATTAGAGGCACTGC
Su. murinus FMNH 134811              ??????????CCTGTTTACCAAAAACATCACCTCTAGCATTATCAGTATTAGAGGCACTGC
Su. lixus FMNH 155667                ?????CCCGCCTGTTTACCAAAAACATCACCTCTAGCATTACTAGTATTAGAGGCACTGC
Su. remyi AF274550                   ????????????????????????????????????TAGCATAACAAGTATTAGAGGCACTGC
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Su. sp FMNH 160185                   ??AACCCCGCCTGTTTACCAAAAACATCACCTCTAGCATATCAAGTATTAGAGGCACTGC
Su. sp FMNH 162147                   ??????????????????????AAACATCACCTCTAGCATATCAAGTATTAGAGGCACTGC
Su. sp FMNH 155482                   ???????CGCCTGTTTACCAAAAACATCACCTCTAGCATTACTAGTATTAGAGGCACTGC
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Sy. howelli FMNH 168165              AAAACCCCGCCTGTTTACCAAAAACATCACCTCTAGCATTACCAGTATTAGAGGCACTGC
Sy. howelli FMNH 158308              AAAACCCCGCCTGTTTACCAAAAACATCACCTCTAGCATTACCAGTATTAGAGGCACTGC
Sy. howelli FMNH 161223              AAAACCCCGCCTGTTTACCAAAAACATCACCTCTAGCATTACCAGTATTAGAGGCACTGC
Sy. howelli FMNH 166780              ?AAACCCCGCCTGTTTACCAAAAACATCACCTCTAGCATTACCAGTATTAGAGGCACTGC
Sy. howelli FMNH 150023              ??AACCCCGCCTGTTTACCAAAAACATCACTTCTAGCATTAACAGTATTAGAGGCACTGC
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Sy. johnstoni AY017312               ????????????????????????????????CACTTCTAGCATTAATAGTATTAGAAGCACTGC
Sy. johnstoni AY017315               ????????????????????????????????CACTTCTAGCATTAATAGTATTAGAAGCACTGC
Sy. johnstoni AF252524               ????????????????????????????????CACTTCTAGCATTAATAGTATTAGAAGCACTGC
Sy. johnstoni AY017321               ????????????????????????????????CACTTCTAGCATTAATAGTATTAGAAGCACTGC
Sy. lunaris FMNH 144229              CAAACCCCGCCTGTTTACCAAAAACATCACTTCTAGCATTAATAGTATTAGAAGCACTGC
Sy. megalura AF274555                ????????????????????????????????????AGCATTACCAGNATTAGAGGCACTGC
Sy. megalura FMNH 150379             CAAACCCCGCCTGTTTACCAAAAACATCACCTCTAGCATTACCAGTATTAGAGGCACTGC
Sy. ollula FMNH 162189               CAAACCCCGCCTGTTTACCAAAAACATCACCTCTAGCATAATAAGTATTAGAGGCACTGC
Sy. ollula AF253953                  ????????????????????????????????CACCTCTAGCATAATAAGTATTAGAGGCACTGC
Sy. ollula AF253967                  ????????????????????????????????CACCTCTAGCATAATAAGTATTAGAGGCACTGC
Sy. ollula AF253957                  ????????????????????????????????CACCTCTAGCATAATAAGTATTAGAGGCACTGC
Sy. ollula AF253951                  ????????????????????????????????CACCTCTAGCATAATAAGTATTAGAGGCACTGC
Sy. ollula AF253960                  ????????????????????????????????CACCTCTAGCATAATAAGTATTAGAGGCACTGC
Sy. vulcanorum FMNH 144237           TAAACCCCGCCTGTTTACCAAAAACATCACCTCTAGCATTACTATTATTAGAGGCACTGC
Sy. vulcanorum FMNH 144240           TAAACCCCGCCTGTTTACCAAAAACATCACCTCTAGCATTACTATTATTAGAGGCACTG
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Species	940	999
So. araneus AF274562	CTGCCCAGTGAC-AACT--GTT-TAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Sor. fumidus AF274561	CTGCCCAGTGAC-AACT--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Co.sp AF274559	CTGCCCAGTGAC-ATAA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Co.phillipsorum FMNH 177632	CTGCCCAGTGAC-ACAA--GTTTAAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Co.phillipsorum FMNH 177689	CTGCCCAGTGAC-ACAA--GTTTAAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
My. geata AF274560	CTGCCCAGTGAC-ACAA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
My. kihaulei FMNH155622	CTGCCCAGTGAC-ACAA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Cr. fuliginosa AF274548	CTGCCCAGTGAC-ATTA--GTT-TAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Cr. goliath AF274539	CTGCCCAGTGAC-ATTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
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Cr. lamottei AF274546	CTGCCCAGTGAC-ATTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Cr. leucodon AF274544	CTGCCCAGTGAC-ATTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Cr. malayana AF274547	CTGCCCAGTGAC-ATTA--GTT-TAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Cr. obscurior AF274545	CTGCCCAGTGAC-ATTA--GTT-TAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
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Cr. roosevelti AF274542	CTGCCCAGTGACAATTA--GTT-TAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Cr. russula AF274543	CTGCCCAGTGAC-ATTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
P. maxima AF274541	CTGCCCAGTGAC-ATTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
P. schoutedeni AF274540	CTGCCCAGTGAC-ATTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
R. suncoides AF274558	CTGCCCAGTGAC-ATTA--GTT-TAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Sc. somereni AF274557	CTGCCCAGTGAC-ATTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Su. dayi AF274554	CTGCCCAGTGAC-ATCA--GTT-TAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Su. etruscus AF274549	CTGCCCAGTGAC-ATTA--GTT-TAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Su. infinitesimus AF274551	CTGCCCAGTGAC-ATTA--GTT-TAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Su. madagascariensis FMNH 178783	CTGCCCAGTGAC-ATTA--GTT-TAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Su. montanus AF274553	CTGCCCAGTGAC-ATTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Su. murinus AF274552	CTGCCCAGTGAC-ATTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Su. murinus SMG 8677	CTGCCCAGTGAC-ATTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
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Su. lixus FMNH 155667	CTGCCCAGTGAC-ACTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Su. remyi AF274550	CTGCCCAGTGAC-ATTA--GTT-TAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Su. remyi FMNH 162147	CTGCCCAGTGAC-ATTA--GTT-TAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Su. sp FMNH 160185	CTGCCCAGTGAC-ATTA--GTT-TAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Su. sp FMNH 162147	CTGCCCAGTGAC-ATTA--GTT-TAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
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Sy. johnstoni AY017321	CTGCCCAGTGAC-ACTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
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Sy. megalura AF274555	CTGCCCAGTGAC-ATCA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Sy. megalura FMNH150379	CTGCCCAGTGAC-ATCA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Sy. ollula FMNH162189	CTGCCCAGTGAC-ATTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Sy. ollula AF253953	CTGCCCAGTGAC-ATTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Sy. ollula AF253967	CTGCCCAGTGAC-ATTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Sy. ollula AF253957	CTGCCCAGTGAC-ATTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Sy. ollula AF253951	CTGCCCAGTGAC-ATTA--GTT-AAACGGCCGCGGTATTCTGACCGTGCAAAGGTAGCAT	
Sy. ollula AF253960	CTGCCCAGTGAC-ATTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Sy. vulcanorum FMNH 144237	CTGCCCAGTGAC-ACTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Sy. vulcanorum FMNH144240	CTGCCCAGTGAC-ACTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	
Sy. vulcanorum WTS 406	CTGCCCAGTGAC-ACTA--GTT-AAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCAT	

Species	1000	1059
So. araneus AF274562	AATCATTTGTTCTTTAAATAAGGACTTGTATGAATGGCCACACGAGGGTTTAACTGTCTC	
Sor. fumidus AF274561	AATCATTTGTTCCTTAAATAGGGACTTGTATGAATGGCCACACGAGGGTTTAACTGTCTC	
Co.sp AF274559	AATCATTTGTTCCTTAATTAGGGACTAGTATGAACGGCCACACGAGGGTTTAACTGTCTC	
Co.phillipsorum FMNH177632	AATCATTTGTTCCTTAATTAGGGACTAGTATGAATGGCCACACGAGGGTTTAACTGTCTC	
Co.phillipsorum FMNH177689	AATCATTTGTTCCTTAATTAGGGACTAGTATGAATGGCCACACGAGGGTTTAACTGTCTC	
My. geata AF274560	AATCATTTGTTCCTTAATTAGGGACTAGTATGAATGGCCACACGAGGGTTTAACTGTCTC	
My. kihaulei FMNH155622	AATCATTTGTTCCTTAATTAGGGACTAGTATGAATGGCCACACGAGGGTTTAACTGTCTC	
Cr. fuliginosa AF274548	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCACACGAGGGTTTAACTGTCTC	
Cr. goliath AF274539	AATCATTTGTTCCTTAATTAGGGACTTGTATGAACGGCCCCACGAGGGTTTAACTGTCTC	
Cr. hidegardeae FMNH155538	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCCCACGAGGGTTTAACTGTCTC	
Cr. lamottei AF274546	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCCCACGAGGGTTTGACTGTCTC	
Cr. leucodon AF274544	AATCATTTGTTCCTTAATTAGGGACTAGTATGAATGGCCCCACGAGGGTTTAACTGTCTC	
Cr. malayana AF274547	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCCCACGAGGGTTTGACTGTCTC	
Cr. obscurior AF274545	AATCATTTGTTCCCTAATTAGGGACTCGTATGAATGGCCCCACGAGGGTTTAACTGTCTC	
Cr. olivieri FMNH144191	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCTCACGAGGGTTTAACTGTCTC	
Cr. roosevelti AF274542	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCCCACGAGGGTTTAACTGTCTC	
Cr. russula AF274543	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCCCACGAGGGTTTAACTGTCTC	
P. maxima AF274541	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCCCACGAGGGTTTGACTGTCTC	
P. schoutedeni AF274540	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCCCACGAGGGTTTGACTGTCTC	
R. suncoides AF274558	AATCATTTGTTCCTTAATTAGGGACTCGTATGAACGGCCACACGAGGGTTTAACTGTCTC	
Sc. somereni AF274557	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCACACGAGGGTTTAACTGTCTC	
Su. dayi AF274554	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCCCACGAGGGTTTAACTGTCTC	
Su. etruscus AF274549	AATCATTTGTTCCTTAATTAGGGACTTGTATGAAAGGCTCCACGAGGGTTTAACTGTCTC	
Su. infinitesimus AF274551	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCCCACGAGGGTTTGACTGTCTC	
Su. madagascariensis FMNH 178783	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCTTCACGAGGGTTTAACTGTCTC	
Su. montanus AF274553	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCTCACGAGGGTTTAACTGTCTC	
Su. murinus AF274552	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCTCACGAGGGTTTAACTGTCTC	
Su. murinus SMG 8677	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCTCACGAGGGTTTAACTGTCTC	
Su. murinus FMNH 134811	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCTCACGAGGGTTTAACTGTCTC	
Su. lixus FMNH 155667	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCCCACGAGGGTTTGACTGTCTC	
Su. remyi AF274550	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCCCACGAGGGTTTAACTGTCTC	
Su. remyi FMNH 162147	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCCCACGAGGGTTTAACTGTCTC	
Su. sp FMNH 160185	AATCATTTGTTCCTTAATTAGGGACTTGTATGAACGGCCCCACGAGGGTTTAACTGTCTC	
Su. sp FMNH 162147	AATCATTTGTTCCTTAATTAGGGACTTGTATGAACGGCCCCACGAGGGTTTAACTGTCTC	
Su. sp FMNH 155482	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCCCACGAGGGTTTGACTGTCTC	
Su. varilla FMNH 168107	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCTCACGAGGGTTTAACTGTCTC	
Sy. cf konganensis AF27455656	AATCATTTGTTCCTTAATTAGGGACTTGTATGAAGGGCCACACGAGGGTTTGACTGTCTC	
Sy. granti FMNH 144219	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCACACGAGGGTTTAACTGTCTC	
Sy. howelli FMNH 168165	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCTCACGAGGGTTTAACTGTCTC	
Sy. howelli FMNH 158308	AATCATTTGTTCCTTAATTAGGGACTTGCATGAATGGCCTCACGAGGGTTTAACTGTCTC	
Sy. howelli FMNH 161223	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCTCACGAGGGTTTAACTGTCTC	
Sy. howelli FMNH 166780	AATCATTTGTTCCTTAATTAGGGACTTGTATGAACGGCCTCACGAGGGTTTGACTGTCTC	
Sy. howelli FMNH 150023	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCTCACGAGGGTTTAACTGTCTC	
Sylvisorex johnstoni AF252518	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCTTGACGAGGGTTTAACTGTCTC	
Sy. johnstoni AY017312	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCTTAACGAGGGTTTAACTGTCTC	
Sy. johnstoni AY017315	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCTTGACGAGGGTTTAACTGTCTC	
Sy. johnstoni AF252524	AATCATTTGTTCCTTAATTAGGGACTTGCATGAATGGCTTGACGAGGGTTTAACTGTCTC	
Sy. johnstoni AY017321	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCTTGACGAGGGTTTAACTGTCTC	
Sy. lunaris FMNH 144229	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCTCCACGAGGGTTTAACTGTCTC	
Sy. megalura AF274555	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCACACGAGGGTTTAACTGTCTC	
Sy. megalura FMNH 150379	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCACACGAGGGTTTAACTGTCTC	
Sy. ollula FMNH 162189	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCACACGAGGGTTTAACTGTCTC	
Sy. ollula AF253953	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCACACGAGGGTTTAACTGTCTC	
Sy. ollula AF253967	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCACACGAGGGTTTAACTGTCTC	
Sy. ollula AF253957	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCACACGAGGGTTTAACTGTCTC	
Sy. ollula AF253951	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCACACGAGGGTTTAACTGTCTC	
Sy. ollula AF253960	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCACACGAGGGTTTAACTGTCTC	
Sy. vulcanorum FMNH 144237	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCCCACGAGGGTTTAACTGTCTC	
Sy. vulcanorum FMNH 144240	AATCATTTGTTCCTTAATTAGGGACTTGTATGAATGGCCCCACGAGGGTTTAACTGTCTC	

Species	1059	1119
So. araneus AF274562	TTATTTCTAATCAGTGAAATTGACCTTTCCGTGAAGAGGCGGAAATAATCTAATAAGACG	
Sor. fumidus AF274561	TTACTTCCAATCAGTGAAATTGACCTCTCCGTGAAGAGGCGGAAATAAATGAATAAGACG	
Co.sp AF274559	TTATTTCTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAAAAAATAAGACG	
Co.phillipsorum FMNH177632	TTATTTCTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATACTCAAACAAGACG	
Co.phillipsorum FMNH177689	TTATTTCTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATACTCAAACAAGACG	
My. geata AF274560	TTATTTCTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAACAAAATAAGACG	
My. kihaulei FMNH155622	TTATTTCTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAACAAAATAAGACG	
Cr. fuliginosa AF274548	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAGAAAAATAAGACG	
Cr. goliath AF274539	TTACTTTTAATCAGTGAAATTGACCTCCCCGTGAAGAGGCGGGAATACAAAAATAAGACG	
Cr. hidegardeae FMNH155538	TTACTTTCAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATATAAAAATAAGACG	
Cr. lamottei AF274546	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATACAAAAATAAGACG	
Cr. leucodon AF274544	TTACTTTCAATCAGTGAAATTGACCTCCCCGTGAAGAGGCGGGGATATAAAAATAAGACG	
Cr. malayana AF274547	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATATAAAAATAAGACG	
Cr. obscurior AF274545	TTACTTTCAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAGAAAATAAGACG	
Cr. olivieri FMNH144191	TTACTTTTGATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATACAAGAATAAGACG	
Cr. roosevelti AF274542	TTACTTCTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATATAAAAATAAGACG	
Cr. russula AF274543	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATATATAAATAAGACG	
P. maxima AF274541	TTACTTTTGATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGAAATAAAAAAATAAGACG	
P. schoutedeni AF274540	TTACTTTTGATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATACAAAAATAAGACG	
R. suncoides AF274558	TTACTTTTAATCAGTGAAATTGACCTCCCCGTGAAGAGGCGGGGATGCAAAAATAAGACG	
Sc. somereni AF274557	TTACTTTCAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAAAAAATAAGACG	
Su. dayi AF274554	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATATTAAAATAAGACG	
Su. etruscus AF274549	TTACTTTCAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAAAAAATAAGACG	
Su. infinitesimus AF274551	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAAATAATAAGACG	
Su. madagascariensis FMNH 178783	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAAAAAATAAGACG	
Su. montanus AF274553	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATACTAAAATAAGACG	
Su. murinus AF274552	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATACTAAAATAAGACG	
Su. murinus SMG 8677	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATACTAAAATAAGACG	
Su. murinus FMNH 134811	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATACTAAAATAAGACG	
Su. lixus FMNH 155667	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGACGAGGCGGGAATAATATAATAAGACG	
Su. remyi AF274550	TTACTTTCAATCAGTGAAATTGACTTTCCCGTGAAGAGGCGGGAATAAAACAATAAGACG	
Su. remyi FMNH 162147	TTACTTTCAATCAGTGAAATTGACTTTCCCGTGAAGAGGCGGGAATAAAACAATAAGACG	
Su. sp FMNH 160185	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAAACAATAAGACG	
Su. sp FMNH 162147	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAAACAATAAGACG	
Su. sp FMNH 155482	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGACGAGGCGGGAATAATATAATAAGACG	
Su. varilla FMNH 168107	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAATTAATAAGACG	
Sy. cf konganensis AF27455656	TTACTTTTAATCAGTGAAATTGACCTCCCCGTGAAGAGGCGGGAATGAAAAAATAAGACG	
Sy. granti FMNH 144219	TTACTTTTAATCAGTGAAATTGACCTCCCCGTGAAGAGGCGGGAATAAAAAAATAAGACG	
Sy. howelli FMNH 168165	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAAATAATAAGACG	
Sy. howelli FMNH 158308	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAAATAATAAGACG	
Sy. howelli FMNH 161223	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGAAATAAAATAATAAGACG	
Sy. howelli FMNH 166780	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAAACAATAAGACG	
Sy. howelli FMNH 150023	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAAATAATAAGACG	
Sy. johnstoni AF252518	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAATTAATAAGACG	
Sy. johnstoni AY017312	TTACTTTTGATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAATTAATAAGACG	
Sy. johnstoni AY017315	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAATTAATAAGACG	
Sy. johnstoni AF252524	TTACTTTCAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAATTAATAAGACG	
Sy. johnstoni AY017321	TTACTTTTMATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAATTAATAAGACG	
Sy. lunaris FMNH 144229	TTACTTTTAATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAACAAATAAGACG	
Sy. megalura AF274555	TTACTTTTTATCAGTGAAATTGACCTCCCCGTGAAGAGGCGGGGATATAACAATAAGACG	
Sy. megalura FMNH 150379	TTACTTTTTATCAGTGAAATTGACCTCCCCGTGAAGAGGCGGGGATATAACAATAAGACG	
Sy. ollula FMNH162189	TTACTTTTAATCAGTGAAATTGACCTCCCCGTGAAGAGGCGGGAATAAAAAAATAAGACG	
Sy. ollula AF253953	TTACTTTTAATCAGTGAAATTGACCTCCCCGTGAAGAGGCGGGAATAAAAAAATAAGACG	
Sy. ollula AF253967	TTACTTTTAATCAGTGAAATTGACCTCCCCGTGAAGAGGCGGGAATAAAAAAATAAGACG	
Sy. ollula AF253957	TTACTTTTAATCAGTGAAAYTGACCTCCCCGTGMAGAGGCGGGAATAAAAAAATAAGACG	
Sy. ollula AF253951	TTACTTTTAATCAGTGAAATTGACCTCCCCGTGAAGAGGCGGGGATAAAAAAATAAGACG	
Sy. ollula AF253960	TTACTTTTGATCAGTGAAATTGACCTCCCCGTGAAGAGGCGGGAATAAAAAAATAAGACG	
Sy. vulcanorum FMNH 144237	TTACTTTCAATCAATGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAAATAATAAGACG	
Sy. vulcanorum FMNH 144240	TTACTTTCAATCAATGAAATTGACCTTCCCGTGAAGAGGCGGGAATAAAATAATAAGACG	

Species	1120	1179
So. araneus AF274562	AGAAGACCCTATGGAGCTTTAATTGACCTAGTCCAACAGAATAGTACTTAACTCCACCAA	
Sor. fumidus AF274561	AGAAGACCCTATGGAGCTTTAATTTACTTAATTCAACAGGAATTGATTCATTACCCACCA	
Co.sp AF274559	AGAAGACCCTATGGAGCTTAAATTATATAACTCAATAATAATAACTAAACTCCTACATAG	
Co.phillipsorum FMNH 177632	AGAAGACCCTATGGAGCTTAAATTAAATAACTCAACAAAATAATATTTACCCAATAGGAA	
Co.phillipsorum FMNH 177689	AGAAGACCCTATGGAGCTTAAATTAAATAACTCAACAAAATAATATTTACCCAATAGGAA	
My. geata AF274560	AGAAGACCCTATGGAGCTTAAATTATATAACCCAATAATAATAACCATATCTCCTAATTC	
My. kihaulei FMNH 155622	AGAAGACCCTATGGAGCTTAAATTATATAACCCAATAATAATAACCATATCTCCTAATTC	
Cr. fuliginosa AF274548	AGAAGACCCTATGGAGCTTAAATTTAAGTGTTCAACAAAATTTAAACTCAATCCAACAGG	
Cr. goliath AF274539	AGAAGACCCTATGGAGCTTAAATTAATATGTTCAACAGAATTTAATTATAATCCAATAGG	
Cr. hidegardeae FMNH 155538	AGAAGACCCTATGGAGCTTAAATTAATATGCTCAACAAAATTTAAATTCAACCCAACAGG	
Cr. lamottei AF274546	AGAAGACCCTATGGAGCTTTAATTAACGTGTTCAACAGAATTTAAATTTCATCCAACAGG	
Cr. leucodon AF274544	AGAAGACCCTATGGAGCTTAAATTAAATATCCAACAAAACTAAACTTAATCCAACAGGAA	
Cr. malayana AF274547	AGAAGACCCTATGGAGCTTAAATTAAAATGTTCAACAAAATTTTAACTTAATCCAACAGG	
Cr. obscurior AF274545	AGAAGACCCTATGGAGCTTAAATTAGATAGTCCAACAAAATCCAAACTTAATCCAACAGG	
Cr. olivieri FMNH144191	AGAAGACCCTATGGAGCTTAAATTAATATGTTCAACAGAATTTAATTATAGCCCAATAGG	
Cr. roosevelti AF274542	AGAAGACCCTATGGAGCTTAAATTAACGTGTCCAACAAAACTTAATATTAACCCAACAGG	
Cr. russula AF274543	AGAAGACCCTATGGAGCTTAAATTAACGTGTCCAACAAAATCTATTCTTAATCCAACAGG	
P. maxima AF274541	AGAAGACCCTATGGAGCTTAAATTAATATGTTCAACAAAATTAAAATCTAATCCAACAGG	
P. schoutedeni AF274540	AGAAGACCCTATGGAGCTTAAATTAATATGTTCAACAAAATTTAAATTTAACCCAACAGG	
R. suncoides AF274558	AGAAGACCCTATGGAGCTTAAATTAAATTATCCAACAAGCCCAGACCTTAACCCAACAGG	
Sc. somereni AF274557	AGAAGACCCTATGGAGCTTAAATTAAACTATTCAATAATTTAAACATAATTCCCACAGGA	
Su. davi AF274554	AGAAGACCCTATGGAGCTTTAATTTATTTGTCCAACAAAATCCTAATTTAATCCAACAGG	
Su. etruscus AF274549	AGAAGACCCTATGGAGCTTAAATTAAATTATCTAATAAATTAAAACTTAATCCCNTAGGC	
Su. infinitesimus AF274551	AGAAGACCCTATGGAGCTTTAATTAATTTGTCTATTATACTTAAAATTCAACTCAACAGA	
Su. madagascariensis FMNH 178783	AGAAGACCCTATGGAGCTTAAATTAAATTATCTAATAAATTAAAACTTAATCCAATAGGC	
Su. montanus AF274553	AGAAGACCCTATGGAGCTTAAATTAAATTATCCAACAAACTTAACTTAAATCCAACAGGG	
Su. murinus AF274552	AGAAGACCCTATGGAGCTTAAATTAAATTATCCAACAAACCTAACTTAAATCCAACAGGA	
Su. murinus SMG 8677	AGAAGACCCTATGGAGCTTAAATTAAATTATCCAACAAACCTAACTTAAATCCAACAGGA	
Su. murinus FMNH 134811	AGAAGACCCTATGGAGCTTAAATTAAATTATCCAACAAACCTAACTTAAATCCAACAGGA	
Su. lixus FMNH 155667	AGAAGACCCTATGGAGCTTAAATTAAATTATCCAATATGATTAAATCTTTATAAACCTAG	
Su. remyi AF274550	AGAAGACCCTATGGAGCTTAAATTAACTTGTCTATTACACCTAAACTCAATTCAACAGAA	
Su. remyi FMNH 162147	AGAAGACCCTATGGAGCTTAAATTAACTTGTCTATTACACCTAAACTCAATTCAACAGAA	
Su. sp FMNH 160185	AGAAGACCCTATGGAGCTTAAATTAATTTGTCTACTATACTTTTAATATATTTCAACAGA	
Su. sp FMNH 162147	AGAAGACCCTATGGAGCTTAAATTAATTTGTCTACTATACTTTTAATATATTTCAACAGA	
Su. sp FMNH 155482	AGAAGACCCTATGGAGCTTAAATTAAATTATCCAATATGATTAAATCTTTATAAACCTAG	
Su. varilla FMNH 168107	AGAAGACCCTATGGAGCTTAAATCAACTTGTCCAATATAACTAAACTTAAACCCAACAGG	
Sy. cf konganensis AF27455656	AGAAGACCCTATGGAGCTTAAATTAAATTACCCAATAATGGACATACAATTCCGATAGGA	
Sy. granti FMNH 144219	AGAAGACCCTATGGAGCTTAAATTAAATTACCCAATAATATAAAGATAACCCCGACAGGA	
Sy. howelli FMNH 168165	AGAAGACCCTATGGAGCTTAAATTAACTTGTCCAACATAACTAAACTTAAAATCCAACAG	
Sy. howelli FMNH 158308	AGAAGACCCTATGGAGCTTAAATTAACTTGTCCAACATAGCT-AGCTCTAAATCCAACAG	
Sy. howelli FMNH 161223	AGAAGACCCTATGGAGCTTAAATTAACTTGTCCAACATAACTAAACTTAAAATCCAACAG	
Sy. howelli FMNH 166780	AGAAGACCCTATGGAGCTTAAATTAACTTGCCCAACATGATTAAACTTAAAATCCAACAG	
Sy. howelli FMNH 150023	AGAAGACCCTATGGAGCTTAAATTAACTTGTCCAACATGACTAAACTTTAAATCCAACAG	
Sy. johnstoni AF252518	AGAAGACCCTATGGAGCTTAAATTGAATTATTCAAATAACCTAAATTCTTNCCCACGGGG	
Sy. johnstoni AY017312	AGAAGACCCTATGGAGCTTAAATTAAATTATTCAAATAACCTAAAATTTTTCCCCACGGG	
Sy. johnstoni AY017315	AGAAGACCCTATGGAGCTTAAATTAAATTATTCAATTTATATAAATTTTTACCTACGGGG	
Sy. johnstoni AF252524	AGAAGACCCTATGGAGCTTAAATTAAATTATTCAATTTATATAAATTTTTACCTACGGGA	
Sy. johnstoni AY017321	AGAAGACCCTATGGAGCTTAAATTGAATTATTCAAATAACCTAAATTCTTCCCCACGGGG	
Sy. lunaris FMNH 144229	AGAAGACCCTATGGAGCTTAAATTAAATTACCCAAATAAACTTAAAAATTTTCTATTAGA	
Sy. megalura AF274555	AGAAGACCCTATGGAGCTTAAATTGACCTGCCCAACAAACCTAAAAGCAACCCGACAGGA	
Sy. megalura FMNH 150379	AGAAGACCCTATGGAGCTTAAATTTACTTGCCCAACAAACCTAAAGTCAATCCAACAGGA	
Sy. ollula FMNH 162189	AGAAGACCCTATGGAGCTTAAATTAAATTACCCAATAATATAAAGATAACCCCGACAGGA	
Sy. ollula AF253953	AGAAGACCCTATGGAGCTTAAATTAAATTACCCAATAATATAAAGATAACCCCGACAGGA	
Sy. ollula AF253967	AGAAGACCCTATGGAGCTTRAATTAAATTATCCAATAATATAAAAATGACCCCGACAGGA	
Sy. ollula AF253957	AGAAGACCCTATGGAGCTTWAATTWAATTACCCAATAATATTAAGATAACCCCGACAGGA	
Sy. ollula AF253951	AGAAGACCCTATGGAGCTTAAATTAAATTATCCAATAATATAAAAATGACCCCGACAGGA	
Sy. ollula AF253960	AGAAGACCCTATGGAGCTTAAATTAAATTACCCAGTAACATAAAAATAAACCCGACAGGA	
Sy. vulcanorum FMNH 144237	AGAAGACCCTATGGAGCTTTAATTTTATTGTTCAACATAACCAAAGTATACCCCAACAGG	
Sy. vulcanorum FMNH 144240	AGAAGACCCTATGGAGCTTTAATTTTATTGTTCAACATAACCAAAGTATACCCCAACAGG	

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Species                          1180                                                                1239
                                  |                                                                  |
So. araneus AF274562             GGACTAACAAACATTCTAAC-------TGGGCTATAAATTTCGGTTGGGGTGACCTCGGA
Sor. fumidus AF274561            GGAATAATAAACACCCTGTA-------TGAATTAAAAATTTTGGTTGGGGTGACCTCGGA
Co.sp AF274559                   GAATTATAAAATCAATTA---------TCTCAGTTAGATTTCGGTTGGGGTGACCTCGGA
Co.phillipsorum FMNH 177632      CTAAACCTAAACCTTGACA--------TGAGTTATAAATTTCGGTTGGGGTGACCTCGGA
Co.phillipsorum FMNH 177689      TTAAACCTAAACCTTGACA--------TGAGTTATAAATTTCGGTTGGGGTGACCTCGGA
My. geata AF274560               AGGGATTAAAAACTAATTATT------TGGATTATAGATTTCGGTTGGGGTGACCTCGGA
My. kihaulei FMNH 155622         AGGGATTAAAAACTAATTATT------TGGATTATAGATTTCGGTTGGGGTGACCTCGGA
Cr. fuliginosa AF274548         AATAATATTATTTTAAC----------TGAACAACAAATTTTGGTTGGGGTGACCTCGGA
Cr. goliath AF274539            GATAAAAATATTCTAAC----------TGAACAATAAATTTCGGTTGGGGTGACCTCGGA
Cr. hidegardeae FMNH 155538      AATAATTATAATTTAAC----------TGAGCAATAAATTTTGGTTGGGGTGACCTCGGA
Cr. lamottei AF274546           AATAAAAACTTTTAAC-----------TGAACAATAAATTTTGGTTGGGGTGACCTCGGA
Cr. leucodon AF274544            TAATGATATTTTAAC------------TGGGTAATAAATTTTGGTTGGGGTGACCTCGGA
Cr. malayana AF274547            AATAACAACATTTTAAC----------TGAACAATAAATTTTGGTTGGGGTGACCTCGGA
Cr. obscurior AF274545           AAATAATAATATTTTAAC---------TGGACAACAAATTTTGGTTGGGGTGACCTCGGA
Cr. olivieri FMNH 144191         AATAAAAATATTCTAAC----------TGAACAATAAATTTTGGTTGGGGTGACCTCGGA
Cr. roosevelti AF274542          AATAATTACATTTTAAC----------TGGACAATAAATTTTGGTTGGGGTGACCTCGGA
Cr. russula AF274543             AAATAAAAATATTTTAAC---------TGGACAACAAATTTTGGTTGGGGTGACCTCGGA
P. maxima AF274541               GATAATAATATTTTAAC----------TGAACAATAAATTTTGGTTGGGGTGACCTCGGA
P. schoutedeni AF274540          AACAATAATATTTTAAC----------TGAACAATAAATTTTGGTTGGGGTGACCTCGGA
R. suncoides AF274558            AATAACTCTATCTTAAC----------TGGATAATAAATTTTGGTTGGGGTGACCTCGGA
Sc. somereni AF274557            ACAAAGACACATTATA-----------TGAATAATAAATTTTGGTTGGGGTGACCTCGGA
Su. dayi AF274554                AACAACAATATTTTAAC----------TGGACAACAAATTTTGGTTGGGGTGACCTCGGA
Su. etruscus AF274549            ATAACAATATATTTAAC----------TAGATAACAAATTTTGGTTGGGGTGACCTCGGA
Su. infinitesimus AF274551       AACAAAATTACGTTAAC----------TAGACAATAAATTTTGGTTGGGGTGACCTCGGA
Su. madagascariensis FMNH 178783 ATAACAACATATTTAAC----------TAGATAACAAATTTTGGTTGGGGTGACCTCGGA
Su. montanus AF274553            ATAAATATGATTTAAC-----------TGGGTAATAAATTTTGGTTGGGGTGACCTCGGA
Su. murinus AF274552             ATAATTAAACTTTAAC-----------TGGATAATAAATTTTGGTTGGGGTGACCTCGGA
Su. murinus SMG 8677             ATAATTAAATTTTAAC-----------TGGATAATAAATTTTGGTTGGGGTGACCTCGGA
Su. murinus FMNH 134811          ATAATTAAACTTTAAC-----------TGGATAATAAATTTTGGTTGGGGTGACCTCGGA
Su. lixus FMNH 155667            TAGGAATAATATTATTTTTAAC-----TGGATAATAAATTTTGGTTGGGGTGACCTCGGA
Su. remyi AF274550               ATAAAACCATGTAAAT-----------TAGACAATAAATTTTGGTTGGGGTGACCTCGGA
Su. remyi FMNH 162147            ATAAAACCATGTAAAT-----------TAGATAATAAATTTTGGTTGGGGTGACCTCGGA
Su. sp FMNH 160185               AATAAAATCATGATAAC----------TAGGCAATAAATTTTGGTTGGGGTGACCTCGGA
Su. sp FMNH 162147               AATAAAATCATGATAAC----------TAGGCAATAAATTTTGGTTGGGGTGACCTCGGA
Su. sp FMNH 155482               TAGGAATAATATTATTTTAAC------TGGATAATAAATTTTGGTTGGGGTGACCTCGGA
Su. varilla FMNH 168107          GACAATACCAATTTAAC----------TGGACAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. cf konganensis AF274556      ATAATATTATATTATA-----------TGAGTAATAGATTTTGGTTGGGGTGACCTCGGA
Sy. granti FMNH 144219           ACAAAACCACATTATA-----------TGGGTAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. howelli FMNH 168165          GAGTAGTATTAATTTAAC---------TGGGCAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. howelli FMNH 158308          GAATAATATTAATTTAAC---------TGGACAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. howelli FMNH 161223          GAGTAATATTAATTTAAC---------TGGACAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. howelli FMNH 166780          GAATAATATTAATTTAAC---------TGGGCAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. howelli FMNH 150023          GAGTAATATTAATTTAAT---------TGGACAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. johnstoni AF252518           CCAACTACACTATACC-----------TGAATAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. johnstoni AY017312           ACTAACTACACTTAAACC---------TGAATAATAAATTT-GGTTGGGGTGACCTCGGA
Sy. johnstoni AY017315           CCAATTCCACAAAATC-----------TGAATAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. johnstoni AF252524           CCAATTCCACAAAATC-----------TGAATAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. johnstoni AY017321           CCAACTACACTATACC-----------TGAATAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. lunaris FMNH 144229          ACTAACATTATTTTATTA----------TGGGTAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. megalura AF274555            ATAATATCATTTTAAC-----------TGGGCAACAAATTTTGGTTGGGGTGACCTCGGA
Sy. megalura FMNH 150379         GCAATACCATTTTAAC-----------TGGGCACCAAATTTTGGTTGGGGTGACCTCGGA
Sy. ollula FMNH162189            ACAAAACCACATTATA-----------TGGGTAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. ollula AF253953              ACAAAACCACATTATA-----------TGGGTAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. ollula AF253967              ACAAAACTACATTATA-----------TGGATAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. ollula AF253957              ACWAAACCACATTATA-----------TGGGTAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. ollula AF253951              ACAAAACTACATTATA-----------TGGATAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. ollula AF253960              ACAAAGCCACATTATA-----------TGGGTAATAAATTTTGGTTGGGGTGACCTCGGA
Sy. vulcanorum FMNH 144237       AATAACACTGGTTTAAC----------TGGACAACAAATTTTGGTTGGGGTGACCTCGGA
Sy. vulcanorum FMNH 144240       AATAACACTGGTTTAAC----------TGGACAACAAATTTTGGTTGGGGTGACCTCGGA
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Species	1240	1299
So. araneus AF274562	GCACAAAATAACCTCCGAGAGCTATCT--ACTAAGACTGA-CAAGTCAAAGTAAATCTTT	
Sor. fumidus AF274561	GTACAAACAAACCTCCGAGATATTTAT--ACCAAGACCCA-CAAGTCAAAGTAACTAATT	
Co.sp AF274559	GTACAAAACAACCTCCGAGAGATTTTAA-ACCAAGATTAA-CAAATCAAAGTAATAAATC	
Co.phillipsorum FMNH 177632	GTATAAAAAACCCTCCGAGAAATTTAA--GCCAAGATTCA-CCAATCAAAGCTAATAATA	
Co.phillipsorum FMNH 177689	GTATAAAAAACCCTCCGAGAAATTTAA--GCCAAGATTCA-CCAATCAAAGCTAATAATA	
My. geata AF274560	GTACAAAATAACCTCCGAGAGATTTAA--ACCAAGATAAA-CAAATCAAAGTAATAAATC	
My. kihaulei FMNH 155622	GTACAAAATAACCTCCGAGAGATTTAA--ACCAAGATAAA-CAAATCAAAGTAATAAATC	
Cr. fuliginosa AF274548	GTATAATAAAACCTCCGAGCAATTTTA--ACTAAGACTCA-CCTGTCAAAGTGATAAAAT	
Cr. goliath AF274539	GTATAACATAACCTCCGAGCAATTAA---ACCAAGACTTA-CCAGTCAAGGCCATATAAC	
Cr. hidegardeae FMNH 155538	GTATAACAGAACCTCCGAGTGATTTTA--ACCAAGACTTA-CCCGTCAAGGTTACGTAAC	
Cr. lamottei AF274546	GTATAACAAAACCTCCAAGCGATTTA---ACCAAGACTCA-CCAGTCAAGGTCATGTAAT	
Cr. leucodon AF274544	GCACAATAAAACCTCCGAGCTATTTT---ACCAAGACTAA-CCCGTCAAGGTTATTTTTT	
Cr. malavana AF274547	GCATAACAAAACCTCCGAGCAGTTTA---ACTTAGACTTA-CTAGTCAAAGTGATATAAT	
Cr. obscurior AF274545	GTATAATAAAACCCCCGAGCTATTTT---TCCAAGACTTA-CCCGTCAAGGTAGTTTAAT	
Cr. olivieri FMNH 144191	GTATAACATAACCTCCGAGCAATTAA---ACCAAGACTTA-CCAGTCAAGGCTATATAAC	
Cr. roosevelti AF274542	GTATAATCAAACCTCCGAGCGATTTA---ACCAAGACTAA-CCCGTCAAGGTTATATAAC	
Cr. russula AF274543	GTATAACAAAACCTCCGAGAGATATTG--ACCAAGACTTA-CCCGTCAAAGTAATATAAT	
P. maxima AF274541	GTACAAAAAAACCTCCGAGCGATTAA---ATCAAGACTTA-CTAGTCAAAATTATGTAAT	
P. schoutedeni AF274540	GCACAACAAAACCTCCGAGCAACTAA---ATCAAGACTTA-CCAGTCAAGATTATACAAT	
R. suncoides AF274558	GCACAACCTAACCTCCGAGCGATTTAA--ATCAAGACTTA-CTTGTCAAAATAACAATAT	
Sc. somereni AF274557	GTATAAAACAACCTCCGAGTAATTTAA--ACCAAGACTCA-CTCGTCAAAGTAATAAAGC	
Su. dayi AF274554	GCATAACTCAACCTCCGAGCAATCAA---ACTAAGACTTA-CCTGTCAAAGCAACATCAC	
Su. etruscus AF274549	GTATAACCCAACCTCCGAGCTATATAA--ACTAAGACTTA-CCTGTCAAAGTGAAATAAC	
Su. infinitesimus AF274551	GAATAGCTCAACCTCCGAGCGACTAAA--ACTAAGACTAA-CCCGTCAAAGTGGTTTTTA	
Su. madagascariensis FMNH 178783	GTATAACCTAACCTCCGAGCTATATAA--ACTAAGACTTA-CCTGTCAAAGTGAAATAAT	
Su. montanus AF274553	GCATAACTCAACCTCCGAGCAGTTAAA--ACTCAGACTTA-CCCGTCAAAGTTGTACTAT	
Su. murinus AF274552	GCATAACTTAACCTCCGAGCAGTTAAA--ACTCAGACTTA-CCCGTCAAAGTGGAACTAT	
Su. murinus SMG 8677	GCATAACTTAACCTCCGAGCAGTTAAA--ACTCAGACTTA-CCCGTCAAAGTGGAATTAT	
Su. murinus FMNH 134811	GCATAACTTAACCTCCGAGCAGTTAAA--ACTCAGACTTA-CCCGTCAAAGTGGAACTAT	
Su. lixus FMNH 155667	GCATAAAATAACCTCCGAGTAATTCA---ACTAAGACTAA-TTCGTCAAAGTGACTTTAC	
Su. remyi AF274550	GAATAACATAACCTCCGAGCGATTTAA--GCTAAGACTAA-CCCGTCAAAGTGATATTAC	
Su. remyi FMNH 162147	GAATAACATAACCTCCGAGCGATTTAA--GCTAAGACTAA-CCCGTCAAAGTGATATTAC	
Su. sp FMNH 160185	GAAAAACCTAACCTCCGAGCGATTTAA--ACTAAGACTAA-CCCGTCAAAGTGATTTAAC	
Su. sp FMNH 162147	GAAAAACCTAACCTCCGAGCGATTTAA--ACTAAGACTAA-CCCGTCAAAGTGATTTAAC	
Su. sp FMNH 155482	GCATAAAATAACCTCCGAGTAATTCA---ACTAAGACTAA-TTCGTCAAAGTGACTTTAC	
Su. varilla FMNH 168107	GTATAAACCAGCCTCCGAGAAACTAA---ACTAAGACTAA-CCCGTCAAAGTAATTAAAT	
Sy. cf konganensis AF274556	GAATAACCTAACCTCCGAGCAGTTTAA--ACTGAGACCTA-CTTGTCAAAGTGATTATAT	
Sy. granti FMNH 144219	GCATAACATAACCTCCGAGTAATTTAA--CCAAGACCCAC-TTGTCAAAGTGCTAAAACT	
Sy. howelli FMNH 168165	GTATAAATCAACCTCCGAGAGACTAG---ACTGAGACTAA-TTCGTCAAAGTAATTAAAT	
Sy. howelli FMNH 158308	GTATAAATCAACCTCCGAGAGACTAA---ACTGAGACCAA-TTCGTCGAAGTAATTAAAT	
Sy. howelli FMNH 161223	GTATAAATCAACCTCCGAGAGACTAA---ACTGAGACTAA-TTCGTCAAAGTAATTAAAT	
Sy. howelli FMNH 166780	GTATAAATCAACCTCCGAGAGACTAA---ACTGAGACTAA-TTCGTCGAAGTAATAAAAT	
Sy. howelli FMNH 150023	GTATAAATCAACCTCCGAGAAACTAA---ACTAAGACTAA-TTCGTCGAAGTAATTTAAT	
Sy. johnstoni AF252518	GCACAAAATAACCTCCGAGAACTACA---GCTAAGACTTA-CCCGTCAAAGCACTTTAAA	
Sy. johnstoni AY017312	GCATAAAACAACCTCCGAGAATTACA---GCTAAGACTCA-CCCGTCAAAGCATCTTTAA	
Sy. johnstoni AY017315	GTACAAAACAACCTCCGAGATATACA---GCTAAGACTTA-CCCGTCAAAGCATCTTTTA	
Sy. johnstoni AF252524	GTACAAAACAACCTCCGAGATATACA---GCTAAGACTTA-CCCGTCAAAGCATCTCTTA	
Sy. johnstoni AY017321	GCACAAAATAACCTCCGAGAACTACA---GCTAAGACTTA-CCCGTCAAAGCACTTTAAA	
Sy. lunaris FMNH 144229	GAATAAAATAACCTCCGAGTAATAAA---ACCGAGACCTA-CCAGTCAAAGCAACATTAC	
Sy. megalura AF274555	GTATAACTCAACCTCCGAGTGTTCTAA--ACTAAGACCTA-CATGTCAAAGTGACAACAC	
Sy. megalura FMNH 150379	GCATAACCTAACCTCCGAGTGTTTTAA--ACTAAGACTTA-CATGTCAAAGTGACAGCAC	
Sy. ollula FMNH 162189	GCATAACATAACCTCCGAGTAATTTA---ACCAAGACCCA-CTTGTCAAAGTGCTAAAAC	
Sy. ollula AF253953	GCATAACATAACCTCCGAGTAATTTA---ACCAAGACCCA-CTTGTCAAAGTGCTAAAAC	
Sy. ollula AF253967	GCATAGCATAACCTCCGAGTAATTTA---ACCAAGACCCA-CTTGTCAAAGTGCTAAAAC	
Sy. ollula AF253957	GCATAACAYAACCTCCGAGTAATTTA---ACCAAGACCCA-CTTGTCAAAGTGCTAAAAC	
Sy. ollula AF253951	GCATAACATAACCTCCGAGTAATTTA---ACCAAGACCCA-CTTGTCAAAGTACTAAAAC	
Sy. ollula AF253960	GCATAACATAACCTCCGAGTAATTTA---ACCAAGACCCA-CTTGTCAAAGTGCTAAAAC	
Sy. vulcanorum FMNH 144237	GCATAATAAAACCTCCGAGAAATTAA---GCCAAGACGCA-CCTGTCAAAGCGACTCATC	
Sy. vulcanorum FMNH 144240	GCATAATAAAACCTCCGAGAAATTAA---GCCAAGACGCA-CCTGTCAAAGCGACTCATC	

Species	1300	1359
So. araneus AF274562	ACAAATTGATC-------CAG-TCCCA-CTGATCAACGGAACAAGTTACCCTAGGGATAA	
Sor. fumidus AF274561	CAACTGATC---------CAG-TCTAA-CTGATCAACGGAACAAGTTACCCTAGGGATAA	
Co.sp AF274559	AATTATTGATC-------CAA-TCTAA-TTGATCAACGAAACAAGTTACCCTAGGGATAA	
Co.phillipsorum FMNH 177632	ACAATTGATC--------CAA-TCTTA-TTGATCAACGGAACAAGTTACCCTAGGGATAA	
Co.phillipsorum FMNH 177689	ACAATTGAT---------CCAATCTTA-TTGATCAACGGAACAAGTTACCCTAGGGATAA	
My. geata AF274560	AAACATTGATC-------CAA-TCTAA-TTGATCAACGAAACAAGTTACCCTAGGGATAA	
My. kihaulei FMNH 155622	AAACATTGATC-------CAA-TCTAA-TTGATCAACGAAACAAGTTACCCTAGGGATAA	
Cr. fuliginosa AF274548	TTATTGATC---------CAA-TCAAA-TTGATCAACGAAACAAGTTACCCTAGGGATAA	
Cr. goliath AF274539	TTATTGATC---------CAA-TCTTA-TTGATCAACGAAACAAGTTACCCTAGGGATAA	
Cr. hidegardeae FMNH 155538	TTATTGATC---------CAA-TCATA-TTGATCAACGAAACAAGTTACCCTAGGGATAA	
Cr. lamottei AF274546	TTATTGATC---------CAA-TCTTA-TTGATCAACGAAACAAGTTACCCTAGGGATAA	
Cr. leucodon AF274544	TTTTGATC----------CAA-TCATA-TTGATCAACGAAACAAGTTACCCTAGGGATAA	
Cr. malayana AF274547	CTATTGATC---------CAA-TCATA-TTGATCAACGAAACAAGTTACCCTAGGGATAA	
Cr. obscurior AF274545	TTATTGATC---------CAA-TCATA-TTGATCAACGAAACAAGTTACCCTAGGGATAA	
Cr. olivieri FMNH 144191	TTATTGATC---------CAA-TCTTA-TTGATCAACGAAACAAGTTACCCTAGGGATAA	
Cr. roosevelti AF274542	TTATTGATC---------CAA-TCATA-TTGATCAACGAAACAAGTTACCCTAGGGATAA	
Cr. russula AF274543	TTATTGATC---------CAA-TCATA-TTGATCAACGAAACAAGTTACCCTAGGGATAA	
P. maxima AF274541	TAATTGATC---------CAA-TCATA-TTGATCAACGAAACAAGTTACCCTAGGGATAA	
P. schoutedeni AF274540	TTATTGATC---------CAA-TCATA-TTGATCAACGAAACAAGTTACCCTAGGGATAA	
R. suncoides AF274558	TAATTGATC---------CAA-TCATA-TTGATCAACGGAACAAGTTACCCTAGGGATAA	
Sc. somereni AF274557	CTATTGATC---------CAA-TCATA-TTGATCAACGGAACAAGTTACCCTAGGGATAA	
Su. dayi AF274554	TTATTGATC---------CAA-TCTTA-TTGATCAACGGAACAAGTTACCCTAGGGATAA	
Su. etruscus AF274549	TTATTGATC---------CAA-TCTTA-TTGATCAACGGAATAAGTTACCCTAGGGATAA	
Su. infinitesimus AF274551	ATATTGATC---------CAA-TCATA-TTGATCAACGGAACAAGTTACCCTAGGGATAA	
Su. madagascariensis FMNH 178783	TTATTGATC---------CAA-TCTTA-TTGATCAACGGAATAAGTTACCCTAGGGATAA	
Su. montanus AF274553	CAATTGATC---------CAA-TCTTA-TTGATCAACGGAACAAGTTACCCTAGGGATAA	
Su. murinus AF274552	TAATTGATC---------CAA-TCTTA-TTGATCAACGGAACAAGTTACCCTAGGGATAA	
Su. murinus SMG 8677	TAATTGATC---------CAA-TCTTA-TTGATCAACGGAACAAGTTACCCTAGGGATAA	
Su. murinus FMNH 134811	TAATTGATC---------CAA-TCTTA-TTGATCAACGGAACAAGTTACCCTAGGGATAA	
Su. lixus FMNH 155667	TATTGATC----------CAA-TCATA-TTGATCAACGGACCAAGTTACCCTAGGGATAA	
Su. remyi AF274550	TTATTGATC---------CAA-TCATA-TTGATCAACGGAACAAGTTACCCTAGGGATAA	
Su. remyi FMNH 162147	TTATTGATC---------CAA-TCATA-TTGATCAACGGAACAAGTTACCCTAGGGATAA	
Su. sp FMNH 160185	TTATTGATC---------CAA-TCATA-TTGATCAACGGAACAAGTTACCCTAGGGATAA	
Su. sp FMNH 162147	TTATTGATC---------CAA-TCATA-TTGATCAACGGAACAAGTTACCCTAGGGATAA	
Su. sp FMNH 155482	TATTGATC----------CAA-TCATA-TTGATCAACGGACCAAGTTACCCTAGGGATAA	
Su. varilla FMNH 168107	CTATTGAT----------CCAATCATA-TTGATCAACGGACTAAGTTACCCTAGGGATAA	
Sy. cf konganensis AF274556	TTATTGATC---------CAA-TCATA-TTGATCAACGGAACAAGTTACCCTAGGGATAA	
Sy. granti FMNH 144219	TATTGATC----------CAA-TCAATATTGATCAACGGAACAAGTTACCCTAGGGATAA	
Sy. howelli FMNH 168165	CTATTGATC---------CAA-TCGTA-TTGATCAACGGACTAAGTTACCCTAGGGATAA	
Sy. howelli FMNH 158308	CTATTGATC---------CAA-TCATA-TTGATCAACGGACTAAGTTACCCTAGGGATAA	
Sy. howelli FMNH 161223	CTATTGATC---------CAA-TCATA-TTGATCAACGGACTAAGTTACCCTAGGGATAA	
Sy. howelli FMNH 166780	CTATTGATC---------CAA-TCATA-TTGATCAACGGACTAAGTTACCCTAGGGATAA	
Sy. howelli FMNH 150023	TTATTGATC---------CAA-TCATA-TTGATCAACGGACTAAGTTACCCTAGGGATAA	
Sy. johnstoni AF252518	CTATTGATC---------CAATTCATA-TTGATCAACGGAACAAGTTACCCTAGGGATAA	
Sy. johnstoni AY017312	CTATTGATC---------CAA-TTCATATTGATCAACGGAACAAGTTACCCTAGGGATAA	
Sy. johnstoni AY017315	TTATTGATC---------CAA-TTAAA-TTGATCAACGGATCAAGTTACCCTAGGGATAA	
Sy. johnstoni AF252524	TTATTGATC---------CAA-TTATA-TTGATCAACGGATCAAGTTACCCTAGGGATAA	
Sy. johnstoni AY017321	CTATTGATC---------CAA-TTCATATTGATCAACGGAACAAGTTACCCTAGGGATAA	
Sy. lunaris FMNH 144229	TTATTGATC---------CAA-TCATA-TTGATCAACGGAATAAGTTACCCTAGGGATAA	
Sy. megalura AF274555	TTATTGATC---------CAA-TCTTA-TTGATCAACGGACCAAGTTACCCTAGGGATAA	
Sy. megalura FMNH 150379	TTATTGATC---------CAA-TCTTTATTGATCAACGGACCAAGTTACCCTAGGGATAA	
Sy. ollula FMNH 162189	TTATTGATC---------CAA-TCAATATTGATCAACGGAACAAGTTACCCTAGGGATAA	
Sy. ollula AF253953	TTATTGATC---------CAA-TCAATATTGATCAACGGAACAAGTTACCCTAGGGATAA	
Sy. ollula AF253967	TTATTGATC---------CAA-TCAATATTGATCAACGGAACAAGTTACCCTAGGGATAA	
Sy. ollula AF253957	TTAWTGATC---------CAA-TCMATATTGATCAACGGAACAAGTTACCCTAGGGATAA	
Sy. ollula AF253951	TTATTGATC---------CAA-TCAATATTGATCAACGGAACAAGTTACCCTAGGGATAA	
Sy. ollula AF253960	TTATTGATC---------CAA-TCAATATTGATCAACGGAACAAGTTACCCTAGGGATAA	
Sy. vulcanorum FMNH 144237	TATTGATC----------CAA-TCATA-TTGATCAACGGACTAAGTTACCCTAGGGATAA	
Sy. vulcanorum FMNH 144240	TATTGATC----------CAA-TCATA-TTGATCAACGGACTAAGTTACCCTAGGGATAA	

Species	1359	1419
So. araneus AF274562	CAGCGCAATCCTATTCTAGAGTCCCTATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sor. fumidus AF274561	CAGCGCAATCCTATTCAAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Co.sp AF274559	CAGCGCAATCCTATTCAAGAGTCCTTATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Co.phillipsorum FMNH 177632	CAGCGCAATCCTATTCAAGAGTCCATATCGCCAAT--AGGGTTTACGACCTCGATGTTGG	
Co.phillipsorum FMNH 177689	CAGCGCAATCCTATTCAAGAGTCCATATCGCCAAT--AGGGTTTACGACCTCGATGTTGG	
My. geata AF274560	CAGCGCAATCCTATTCAAGAGTCCTTATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
My. kihaulei FMNH 155622	CAGCGCAATCCTATTCAAGAGTCCTTATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Cr. fuliginosa AF274548	CAGCGCAATCCTATTTGAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Cr. goliath AF274539	CAGCGCAATCCTATTTAAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Cr. hidegardeae FMNH 155538	CAGCGCAATCCTATTTGAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Cr. lamottei AF274546	CAGCGCAATCCTATTTGAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Cr. leucodon AF274544	CAGCGCAATCCTATTTAAGAGTTCTTATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Cr. malayana AF274547	CAGCGCAATCCTATTTAAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Cr. obscurior AF274545	CAGCGCAATCCTATTTAAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Cr. olivieri FMNH 144191	CAGCGCAATCCTATTTAAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Cr. roosevelti AF274542	CAGCGCAATCCTATTTAAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Cr. russula AF274543	CAGCGCAATCCTATTTAAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
P. maxima AF274541	CAGCGCAATCCTATTTGAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
P. schoutedeni AF274540	CAGCGCAATCCTATTTGAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
R. suncoides AF274558	CAGCGCAATCCTATTTAAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sc. somereni AF274557	CAGCGCAATCCTATTTAAGAGTTCACATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Su. dayi AF274554	CAGCGCAATCCTATTTAAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Su. etruscus AF274549	CAGCGCAATCCTATTTAAGAGTTCATATCGWCAAT--AGGGTTTACGACCTCGATGTTGG	
Su. infinitesimus AF274551	CAGCGCAATCCTATTTAAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Su. madagascariensis FMNH 178783	CAGCGCAATCCTATTTAAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Su. montanus AF274553	CAGCGCAATCCTATTTGAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Su. murinus AF274552	CAGCGCAATCCTATTTGAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Su. murinus SMG 8677	CAGCGCAATCCTATTTGAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Su. murinus FMNH 134811	CAGCGCAATCCTATTTAAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Su. lixus FMNH 155667	CAGCGCAATCCTATTTAAGAGTTCCTATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Su. remyi AF274550	CAGCGCAATCCTATTTAAGAGTCCCTATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Su. remyi FMNH 162147	CAGCGCAATCCTATTTAAGAGTCCCTATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Su. sp FMNH 160185	CAGCGCAATCCTATTTAAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Su. sp FMNH 162147	CAGCGCAATCCTATTTAAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Su. sp FMNH 155482	CAGCGCAATCCTATTTAAGAGTTCCTATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Su. varilla FMNH 168107	CAGCGCAATCCTATTTAAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. cf konganensis AF274556	CAGCGCAATCCTATTTAAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. granti FMNH 144219	CAGCGCAATCCTATTTGAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. howelli FMNH 168165	CAGCGCAATCCTATTTAAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. howelli FMNH 158308	CAGCGCAATCCTATTTAAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. howelli FMNH 161223	CAGCGCAATCCTATTTAAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. howelli FMNH 166780	CAGCGCAATCCTATTTAAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. howelli FMNH 150023	CAGCGCAATCCTATTTAAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. johnstoni AF252518	CAGCGCAATCCTATTTAAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. johnstoni AY017312	CAGCGCAATCCTATTTAAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. johnstoni AY017315	CAGCGCAATCCTATTTAAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. johnstoni AF252524	CAGCGCAATCCTATTTAAGAGTTCGTATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. johnstoni AY017321	CAGCGCAATCCTATTTAAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. lunaris FMNH 144229	CAGCGCAATCCTATTCAAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. megalura AF274555	CAGCGCAATCCCATTTAAGAGTTCATATCGCCAAT--GGGGTTTACGACCTCGATGTTGG	
Sy. megalura FMNH 150379	CAGCGCAATCCCATTTAAGAGTTCATATCGCCAAT--GGGGTTTACGACCTCGATGTTGG	
Sy. ollula FMNH 162189	CAGCGCAATCCTATTTGAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. ollula AF253953	CAGCGCAATCCTATTTGAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. ollula AF253967	CAGCGCAATCCTATTTGAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. ollula AF253957	CAGCGCAATCCTATTTGAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. ollula AF253951	CAGCGCAATCCTATTTGAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. ollula AF253960	CAGCGCAATCCTATTTGAGAGTCCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. vulcanorum FMNH 144237	CAGCGCAATCCTATTTAAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	
Sy. vulcanorum FMNH 144240	CAGCGCAATCCTATTTAAGAGTTCATATCGACAAT--AGGGTTTACGACCTCGATGTTGG	

Species	1420	1480
So. araneus AF274562	ATCAGGACATCCCAATGGTGCAGCAGCTATTAAAGGTTCGTTTGT????????????????	
Sor. fumidus AF274561	ATCAGGACATCCCAATGGTGCAGCAGCTATTAATGGTTCGTTTGT????????????????	
Co.sp AF274559	ATCAGGACATCCTAATGGTGCAGCAGCTATTAATGGT????????????????????????	
Co.phillipsorum FMNH 177632	ATCAGGACATCCTAATGGTGCAGCAGCTATTAACGGTTCGTTTGTTCAACGATTAAAGTCC	
Co.phillipsorum FMNH 177689	ATCAGGACATCCTAATGGTGCAGCAGCTATTAACGGTTCGTTTGTTCAACGATTAAA????	
My. geata AF274560	ATCAGGACATCCTAATGGTGCAGCAGCTATTAATGGTTCGTTTGTTCAACG??????????	
My. kihaulei FMNH 155622	ATCAGGACATCCTAATGGTGCAGCAGCTATTAATGGTTCGTTTGTTCAACGATTAAAGTCC	
Cr. fuliginosa AF274548	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACG??????????	
Cr. goliath AF274539	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGYTCAA????????????	
Cr. hidegardeae FMNH 155538	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACGATTAAAGTCC	
Cr. lamottei AF274546	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACG??????????	
Cr. leucodon AF274544	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACG??????????	
Cr. malayana AF274547	ATCAGGACACCCAAATGGTGTAGCAGCTATTAAAGGTTCGTTTGTTCAACG??????????	
Cr. obscurior AF274545	ATCAGGACCCCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACG??????????	
Cr. olivieri FMNH 144191	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACGATTAAAGTCC	
Cr. roosevelti AF274542	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACG??????????	
Cr. russula AF274543	ATCAGGACATCCAAATGGTGCAACAGCTATTAAAGGTTCGTTTGTTCAACG??????????	
P. maxima AF274541	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACG??????????	
P. schoutedeni AF274540	ATCAGGACACCCAAATGGTGCAGCAGCTATT????????????????????????????	
R. suncoides AF274558	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACG??????????	
Sc. somereni AF274557	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACG??????????	
Su. davi AF274554	ATCAGGACACCCAAATGGTGCAGCAGCTAATAAAGGTTCGTTTGTTCAACG??????????	
Su. etruscus AF274549	ATCAGGACACCCTAATGGTGCAGCAGCT????????????????????????????????	
Su. infinitesimus AF274551	ATCAGGACACCCAGATGGTGCAGAAGCTATTAAAGGTTCGTTTGTTCAACG??????????	
Su. madagascariensis FMNH 178783	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACGATTAAAGTCC	
Su. montanus AF274553	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACG??????????	
Su. murinus AF274552	ATCAGGACATCCTAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACG??????????	
Su. murinus SMG 8677	ATCAGGACATCCTAATGGTGCAGCAGCTA????????????????????????????????	
Su. murinus FMNH 134811	ATCAGGACATCCTAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACGATTAAAG???	
Su. lixus FMNH 155667	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACGATTAAAG???	
Su. remyi AF274550	ATCAGGACATCCAAATGGTGTAGAAGCTATTAATGGTTCGTTTGTTCAACG??????????	
Su. remyi FMNH 162147	ATCAGGACATCCAAATGGTGTAGAAGCTATTAATGGTTCGTTTGTTCAACGATTAAAG???	
Su. sp FMNH 160185	ATCAGGACACCCAAATGGTGCAGAAGCTATTAAAGGTTCG??????????????????????	
Su. sp FMNH 162147	ATCAGGACACCCAAATGGTGCAGAAGCTATTAAAGGTTCGTTTGTTCAACGATTAAAGTCC	
Su. sp FMNH 155482	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACGA?????????	
Su. varilla FMNH 168107	ATCAGGACATCCTAATGGTGCAGCAGCTAT-AAAGGTTCGTTTGTTCAACGAT????????	
Sy. cf konganensis AF274556	ATCAGGACATCCTAATGGTGCAGCAGCTATTAATGGTT??????????????????????	
Sy. granti FMNH 144219	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACGATTAAAGTCC	
Sy. howelli FMNH 168165	ATCAGGACATCCTAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACGATTAAAGTCC	
Sy. howelli FMNH 158308	ATCAGGACATCCTAATGGTGCAGCAGCTATTAAGGGTTCGTTTGTTCAACGATTAAAGTCC	
Sy. howelli FMNH 161223	ATCAGGACATCCTAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACGATTAAA????	
Sy. howelli FMNH 166780	ATCAGGACATCCTAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACAATTAAAGTCC	
Sy. howelli FMNH 150023	ATCAGGACATCCCAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACGATTAAAGTCC	
Sy. johnstoni AF252518	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACG??????????	
Sy. johnstoni AY017312	ATCAGGACACCNAAATGGTGCAGCAGCTATT????????????????????????????	
Sy. johnstoni AY017315	ATCAGGACACCCAAATGGTGCAGCAGCTATTAA????????????????????????????	
Sy. johnstoni AF252524	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACG??????????	
Sy. johnstoni AY017321	ATCAGGACACCCAAATGGTGCAGCAGCTATT????????????????????????????	
Sy. lunaris FMNH 144229	ATCAGGACACCCAAATGGTGTAGCAGCTATTAAAGGTTCGTTTGTTCAACGAT????????	
Sy. megalura AF274555	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTT??????????????????	
Sy. megalura FMNH 150379	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACGATTAAAGTC?	
Sy. ollula FMNH 162189	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACGATTAAAGTCC	
Sy. ollula AF253953	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACG??????????	
Sy. ollula AF253967	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTC??????????????	
Sy. ollula AF253957	ATCAGGACACCMWAATGGTGCAGCAGCTATTAAAGGTT????????????????????????	
Sy. ollula AF253951	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTG???????????????????	
Sy. ollula AF253960	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTT???????????????????	
Sy. vulcanorum FMNH 144237	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACGATTAAAGTCC	
Sy. vulcanorum FMNH 144240	ATCAGGACACCCAAATGGTGCAGCAGCTATTAAAGGTTCGTTTGTTCAACGATTAAAGTCC	